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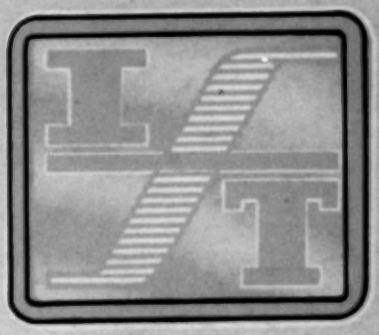

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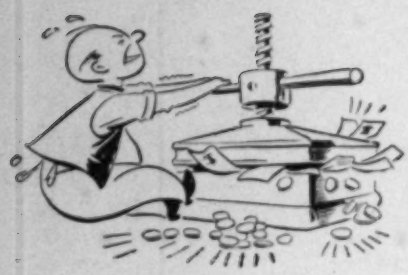
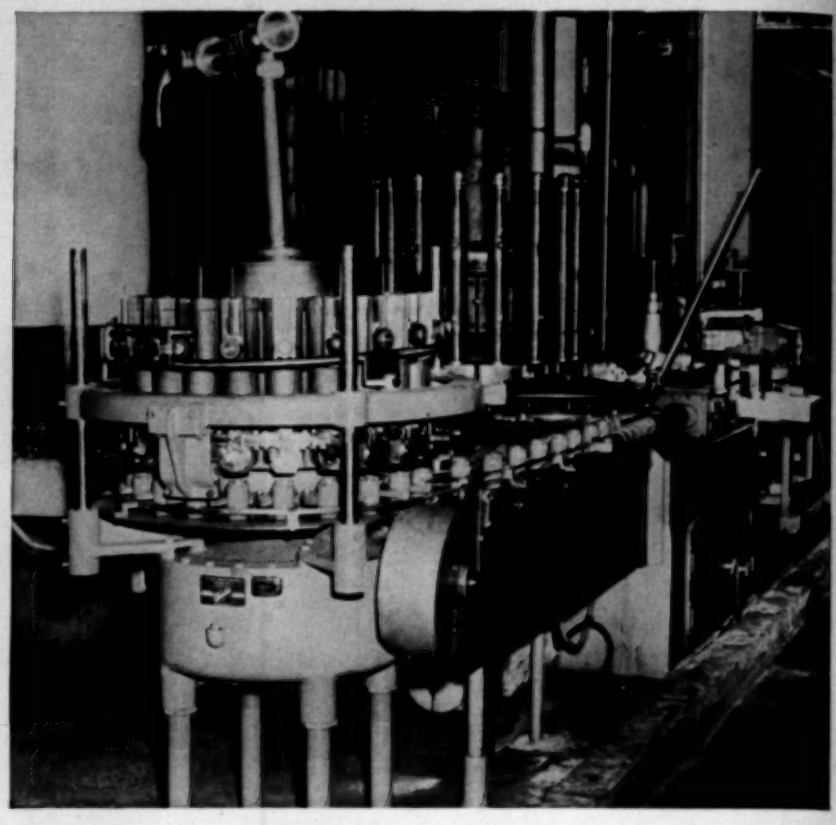
Pfaudler Rotary High-Speed Piston Filler (21 pockets) at Beech-Nut Packing Company, Rochester, N. Y.





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Stream Pollution

In the processing of foods, huge amounts of waste material are accumulated which must be disposed of without creation of a public nuisance. In addition to food processing wastes, there are also tremendous quantities of industrial wastes from chemical plants, textile and paper mills, bleacheries, steel plants, petroleum refineries, strip mines, etc. These will not be discussed here.

Food processing wastes consist of not only solid materials such as trimmings, peels, vines, pods, cobs, etc., but also of liquid effluents made up of cannery washings, cooling and blanching waters, spent lye and acid, and detergent solutions, all of which in general have a high biochemical oxygen demand (B.O.D.). For instance, it has been estimated that the liquid effluent from canning one case of peas has a B.O.D. equivalent to the domestic sewage of three persons. In one year, then, the effluent from the pea pack alone in the United States would be equivalent to the domestic sewage of nearly the entire population of the country.

Solid wastes from fruit and vegetable processing plants amount to over 5 million tons per year—enough to give every man, woman and child 70 pounds of additional garbage in the family garbage pail.

The chemist, in his researches on developing new processes and products, most always creates a waste disposal problem. Very often when he attempts to eliminate one waste he evolves another which may or may not be as bad as the one he sought to eliminate.

What is to be done with all this waste?

In a great many instances liquid effluents are discharged into streams, with little or no treatment. The writer has seen truck-loads of dead fish carted away from a lake upon whose shores a cannery was located. He has seen fine homes vacated because the odors from ponded wastes, carried by prevailing winds, made these home unlivable, even though the ponds were as much as 2 or 3 air miles away. There have been law suits, injunctions, depressed real estate values, hurt feelings, bad tempers and headaches for processors in most every section of the country.

Municipal sewage disposal plants are reluctant, or frankly refuse, to handle canning plant effluents in a great many instances, because most sewage plants do not have the capacity to care for the additional load. Then, too, such effluents, being low in nitrogen, have been known to throw Imhoff tanks out of balance when attempts have been made to handle these wastes in certain municipal plants.

Because it is a cheap method, wastes are sometimes ponded before discharging into streams. This works fairly well where sufficient barren land is available, and where the soil is of the proper texture. But these two conditions are not always present, and nuisances may be created by seepage to a clay stratum where the material is held and subjected to anaerobic decomposition.

Trickling filters have been successful, but their installation is costly, and the canner can only get back his investment by passing the cost on to the consumer, a procedure distasteful to both parties.

In certain sections of the United States, canning and domestic wastes have been disposed of by pumping into deep wells connected with underground streams. One such well, remaining dormant for about a year, suddenly blew up spewing its contents over the scenery, and burned by night with an eerie glow. Before the real significance of the situation had been realized, it was thought an oil gusher had been born. Pumping into deep wells is not without danger of polluting the water supply where this is also obtained from underground streams.

Considerable research has been undertaken over a period of years by State and Federal governments, private organizations and trade associations in the disposition of food processing wastes. Solid residues have been dried for use as feeds; certain types of liquid effluents evaporated to yield molasses, or used as a medium for the production of alcohol or feed yeast. Such uses leave much to be desired. The market for feed is limited and competitive; alcohol and blackstrap prices have dropped so low that profitable or just "break even" alcohol production from waste material is dubious. And the market for feed yeast is limited at prevailing prices. From 12 to 15 million pounds of brewers' dried yeast is now being produced annually, and this amount could easily be increased to 25 million pounds if a ready market could be found for present production. Certain types of waste have also been used to manufacture methane, the gas to be subsequently used as a fuel. Plant investment for such a venture would run pretty high.

The seriousness of stream pollution has been recognized by the 80th Congress which passed the Water Pollution Act. The purpose of the Act is ". . . to recognize, preserve, and protect the primary responsibilities and rights of the States in controlling water pollution, to support and aid technical research to devise and perfect methods of treatment of industrial wastes which are not susceptible to known methods of treatment, and to provide Federal technical services to State and interstate agencies and to industries, and financial aid to State and interstate agencies and to municipalities, in the formulation and execution of their stream pollution abatement programs . . ." Control of the Act is vested in the Surgeon General of the Public Health Service (under the supervision and direction of the Federal Security Administration) and the Federal Works Administration.

Many States have their own water pollution laws, perhaps the most recent being the Water Pollution Control Law of Maryland passed by the Legislature in 1947.

Steam pollution has reached a critical point. What are we going to do about it? It is rather difficult to believe that some one in authority is going to say to an offending food processor: "Either dispose of your waste in an approved sanitary manner, or close your plant." Yet, some day this might happen. The person who issues such an order will undoubtedly lose his job as a reward, but he will have set a precedent which might have far-reaching implications.

Researches in the field of waste disposal may well tax the ingenuity of the technical worker.

HARRY W. VON LOESECKE.

An Analysis of Several Stains Used and Proposed for the Direct Microscopic Enumeration of the Bacteria in Milk

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Several staining procedures either in use or proposed for the direct microscopic determination of bacterial numbers in milk have been studied statistically to determine the ability to yield maximum numbers, and also the deviations. Twelve characteristics looked for in a good stain were evaluated. The North Aniline procedure appears to be the only stain which compares favorably to or excels the Breed stain.

The American Public Health Association in 1915 recognized the method of Prescott and Breed *(10)* as adapted for direct microscopic counts of bacteria in milk by Breed *(1)* as a standard. Since that time it has undergone a complete and thorough investigation, and various authors have suggested alternate procedures. Presumably failure of the staining procedure *(4)* leads to lack of reproducibility and accuracy and this in turn to failure in acceptance as a critical method of examination of milk.

To be employed in staining smears in the direct microscopic enumeration of bacteria in milk, a procedure should enable detection of maximal numbers with a minimum of deviation at all stages of metabolic activity and at all physical conditions normally encountered in milk, and also such that it can be used with assurance of dependability by all laboratory workers in and associated with the dairy industry. Critical evidence of the satisfactory performance of proposed procedures or even the suitability of the present accepted method by comparison with new procedures through statistical evaluation is lacking, although an evaluation unsupported by data is offered by Levine and Black *(4, 5)*.

The general acceptability, expressed through the determination of maximum numbers and their deviations from the mean, and the determination of the value of the stains by an evaluation of desirable characteristics, are reported for several procedures by comparing them to the standard Breed procedure (Standard Methods for the Examination of Dairy Products, ninth edition) and to the North Aniline Oil-Methylene Blue procedure as recommended by North *(9)*. In addition to these, the other procedures studied were those recommended by Broadhurst and Paley *(2)*, Watrous and Doan *(12)*, Gray *(3)* and Mandel *(8)*.

Experimental Procedure

After preliminary studies, 61 producer samples were collected at random from local dairies at the receiving platforms. All samples were promptly iced and at the laboratory six slides were made of each by spreading 0.01 ml. over an area of one square centimeter in accordance with customary procedure. The slides were stained as recommended by the authors. In the computation of the tables and figures, the results obtained previously by staining 100 slides with each of the four stains: Breed, Watrous-Doan, Broadhurst-Paley and Gray on one sample of milk, were included. Counting was done by the strip method of Levowitz *(6)* found acceptable by Watrous and Doan *(12)*.

Results and Discussion

Maximum numbers on a percentage basis and their deviations compared to the standard Breed Stain (Breed's modification of Loeffler's stain*) were computed and are shown in Table 1. With all types of milk,

TABLE 1

Average percent of increase or decrease in count and average percent deviation of five stains for milk films compared with the Breed procedure

Stain Compared with the Breed	Broadhurst-Paley	Gray	Watrous-Doan	North Aniline	Mandel
Average Percent Increase or Decrease	+66	—44	—20	+34	+17
Average Percent Deviation	24	29	25	24	19

of both high and low bacterial counts, the Broadhurst-Paley stain gives a total of 66 percent more bacteria than the Breed; the North stain 34 percent more; the Mandel, 17 percent more; and the Watrous-Doan and Gray procedures give counts materially lower. The average percent deviations vary less widely, but the greatest deviations are shown by the two stains yielding the lowest total counts.

Figure 1 presents the relationship of each stain to the others and the effectiveness of each at lower and higher counts of bacteria in milk. The same order of effective staining is shown. Results with low-count samples show the tendency for all procedures to yield more nearly comparable results. It should be remembered that the direct microscopic method is less effective with low count milks due to the inability to differentiate small differences in size and structure between bacteria and debris. The results obtained have a linear function, the deviation varying with the procedures examined. Larger deviations were found with the higher count samples, confirming the observations of Levine and Black *(4)*. The most effective stain for all types of producer samples appears to be the Broadhurst-Paley procedure, because of its ability to stain maximum numbers. Consistently lower counts are obtained with both Gray and Watrous-Doan stains. The large deviations in the low count milks with the former, and in the high count milks with the latter stain, as well as the general nature of each detract from their value and make their use as a milk stain highly questionable.

*This expression was suggested by Dr. Robert S. Breed at the annual meeting of the Society of American Bacteriologists at Cincinnati, Ohio, May, 1949.

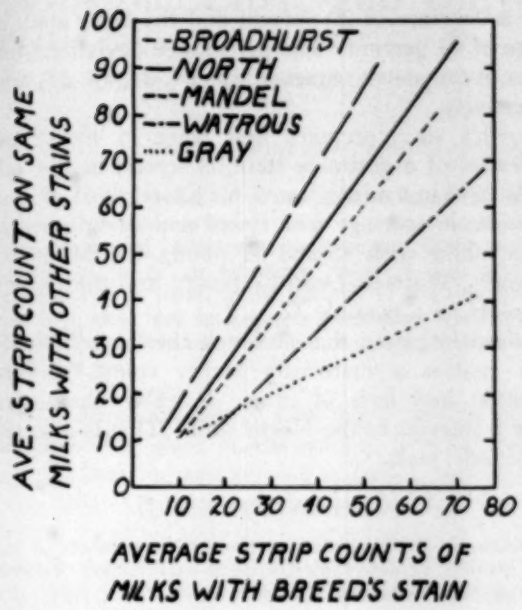

FIG. 1. The bacterial strip counts obtained with several staining procedures compared with those obtained with the Breed procedure on identical milks.

A list of 12 desirable characteristics of a stain was prepared, and an objective evaluation of each stain employed was made. The list is presented in Table 2 and will not be repeated here. For the purpose of preparing a table, numerical values were assigned with the highest value allotted to the stain possessing the desired characteristic to the highest degree. On the basis of this evaluation, the order of general acceptability was (1) the North stain, with a total of 65 points of a possible 72; (2) the Breed stain, 45; (3) the Broadhurst-Paley stain, 42; (4) the Mandel stain, 40; (5) the Watrous-Doan stain, 37; and (6) the Gray stain, 22 points of a possible 72.

The stain proposed by North excels in six of the factors: good differentiation, lack of eyestrain or fatigue, the ability to differentiate isolated bacterial cells, the non-necessity for critical focussing, the brevity of time required for counting, and its usefulness to technicians with vague color distinctions; it places second in five criteria: the ability to present maximal numbers, the fact that there is little tendency toward overstaining (surpassed only by the Watrous-Doan stain), the simplicity of the staining technique, the tendency toward understaining, and minimum of deviation. It is considered third in simplicity of preparation, and this is considered a minor factor for a laboratory technician with experience in the preparation of solutions.

The Breed stain places second in order of preference. It does not excel in any point, nor does it surpass the North stain in any criterion. It is strong in several points of major importance to the technician: fairly good differentiation, moderate in cause of eyestrain or fatigue, in simplicity of the staining technique and in tendency toward understaining. In properly prepared and stained films critical focus is minimal, and since but one dye is involved there is little question of confusion because of vague color distinction. Together with the North stain, little time is required for counting the bacteria of the field, and this is considered an important point in a control laboratory.

The desirability of the Broadhurst-Paley procedure with its ability to detect isolated cells and to present maximal numbers is offset by its wider range of deviation, thereby making it less reliable for individual samples, by the greater care required in distinguishing between cells and debris, by its complexity in use and by the blending of colors. In the hands of careful workers it compares favorably with the Breed stain.

The Gray, Mandel and Watrous-Doan procedures do not yield results comparable to those obtained with the first three procedures, nor, as reference to Table 2 indicates, are they satisfactory in other major respects.

On the basis of results obtained, it was considered advisable to compute the staining procedures using a new basis of comparison, the North stain, since it was the only stain comparable to or more desirable than the Breed stain. Results are given in Table 3. Essentially the same order of staining maximum numbers is demonstrated, with the Broadhurst-Paley stain yielding higher

TABLE 3

Average percent of increase or decrease in count and average percent deviation of five stains for milk films compared with the North Aniline procedure

Stain Compared with the North Aniline	Breed	Broadhurst-Paley	Gray	Watrous-Doan	Mandel
Average Percent Increase or Decrease	—18	+42	—58	—39	—18
Average Percent Deviation	20	22	30	26	23

TABLE 2

*Evaluation of six milk stains according to desirable characteristics**

Characteristics of a Good Staining Procedure	Breed	Broadhurst-Paley	Gray	Watrous-Doan	Mandel	North Aniline
1. Simplicity of preparation	2	1	5	6	3	4
2. Ability to present maximal numbers	3	6	1	2	4	5
3. Tendency toward overstaining	2	4	1	6	3	5
4. Good differentiation	4	5	1	2	3	6
5. Lack of eyestrain or fatigue	4	5	1	2	3	6
6. Ability to see isolated bacteria	4	5	1	2	3	6
7. Need of careful focusing	5	4	1	3	2	6
8. Simplicity of staining technique	4	1	2	6	3	5
9. Time required for counting	5	3	1	2	4	6
10. Usefulness to technicians with vague color distinction	5	1	2	3	4	6
11. Tendency toward understaining	4	3	6	1	2	5
12. Deviation	3	4	1	2	6	5
Total	45	42	22	37	40	65
Order of Preference	2	3	6	5	4	1

*Low figures indicate poor acceptability.

numbers by 42 percent; the Breed and Mandel stains yield 18 percent less, and the Watrous-Doan and Gray stains yield 39 and 58 percent fewer bacteria, respectively. The order of average percent deviation is interchanged with the first two stains, the Breed stain having a deviation of 20 percent and the Broadhurst-Paley 22 percent, with the average deviation of the other stains being greater than the last figure.

Figure 2 demonstrates the comparison of the several stains with the North stain, the counts obtained on each

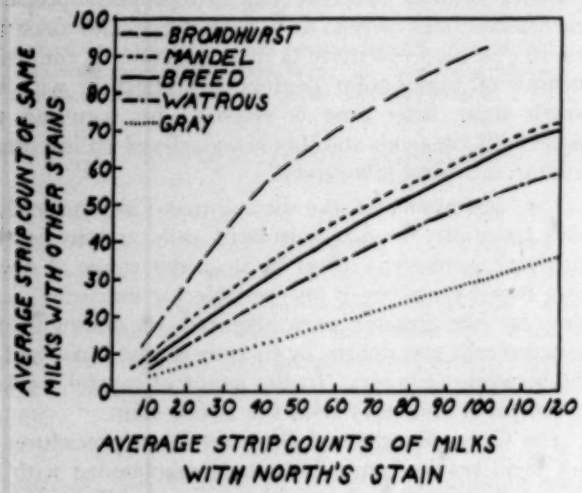

FIG. 2. The bacterial strip counts obtained with several staining procedures compared with those obtained with the North procedure on identical milks.

sample with each stain being plotted against the counts obtained with the North stain. As in the preceding figure, the variation is more pronounced with milks of higher bacterial content. Even at the lower levels, several of the stains yield quite low counts as compared with the North, Breed, and Mandel stains. The tendency to curvature at the higher levels with several of the stains indicates the staining of a larger proportion of physiologically varying bacteria by the North technique than by the others.

Summary

Six stains now in use or proposed for the direct method of counting bacteria in milk were compared statistically to determine maximum numbers and deviations, and the characteristics desired in a good stain were evaluated.

With the Breed stain as the basis of comparison, the Broadhurst-Paley stain gave an average increase in count of 66 percent, the North stain 34 percent, the Mandel stain 17 percent, while the Watrous-Doan stain gave a decrease of 20 percent and the Gray stain a decrease of 44 percent. The percentage deviations for the stains in the above sequence was 24, 24, 19, 25, and 29, respectively.

North's stain occupies first place in six of twelve properties of a desirable stain, is second in five others, and is third in a minor factor, for a total of 65 of possibly 72 points in scoring. The Breed and Broadhurst-Paley stains follow with 45 and 42 points, the Mandel stain 40 points, Watrous-Doan 37 points, and the Gray stain 22 points, of a possible 72.

It is pointed out that although the Broadhurst-Paley stain enables a materially higher count, its greater deviation and lack of other desirable characteristics make it inferior to the North stain. This is also true of the Mandel stain.

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Ascorbic, Citric and Dihydroxymaleic Acids as Antioxidants in Frozen Pack Fruits[a]

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A report is given of a comparative study of the antioxidant value in frozen fruit of crystalline ascorbic, citric and dihydroxymaleic acids. Citric acid proved to be of no significant value in preventing oxidation or in reducing the quantity of ascorbic acid required when use in amounts permitting an edible product. Ascorbic acid was cited, but dihydroxymaleic acid was not.

Many antioxidant agents have been used to prevent browning taking place in preparation, freezing, storage and during thawing and serving of frozen packed fruits *(19, 16, 23)*. Of these agents, ascorbic acid has generally been reported to be the most satisfactory for packing frozen fruit for dessert use and at present is being widely used by commercial operators for this purpose *(3, 11)*. Ascorbic acid is effective in preventing discoloration and retaining the natural fresh characteristic flavor, color and aroma. It has the additional advantage of having vitamin activity. Browning or oxidation, with its resultant loss of flavor or even production of off-flavors as well as creation of an unattractive appearing product, is caused primarily by oxidative enzymes.

Joslyn *(9)* has reported on the chemistry of the enzyme-catalysed oxidative discoloration of fruits. Several investigators [Joslyn and Hohl *(11)*, Wiegand *(19)*, and Woodroof et al. *(23)*] have discussed rather completely the factors involved in enzyme oxidation of fruits and have reviewed present methods of practical control of enzyme activity and prevention of browning in frozen packed fruit.

While ascorbic acid has many advantages as a fruit antioxidant, it still has the one definite disadvantage that it is relatively expensive. Woodroof *(21, 22)* and Luther and Cragwall *(13)* suggested a combination of ascorbic acid and citric acid to prevent browning of prepared fruit. These investigators reported that citric acid inactivated the fruit enzyme and stabilized the ascorbic acid in frozen apple and peach packs. Joslyn and Hohl *(11)* reported that, while citric acid could be substituted satisfactorily for a proportion of the ascorbic acid for color retention in apricots, peaches and nectarines during freezing storage, the fruit frozen with a combination of citric and ascorbic acid did not retain its full fruit flavor as well as when ascorbic acid alone was used. Also, after defrosting the citric acid treated fruit darkened more rapidly. Added acid was not found by these investigators to be objectionable in apricots and peaches. Esselen et al. *(5)* found that citric acid in combination with ascorbic acid was somewhat superior to ascorbic acid alone in retaining color of cold stored fresh apple slices, but was very inferior to SO_2 treated slices.

On the other hand, Dubois *(3)* stated that when citric acid was used in syrup packs in excess of 0.1 to 0.2 percent citric acid of the total weight of the fruit, the fruit becomes tart and furthermore at this strength the citric acid is ineffective in preventing discoloration in packed fruits. Others have obtained similar results [Atkinson, Strachan and Moyls *(1)*, Hoffmann-La Roche *(6)*, and Merck and Co. *(14)*]. These latter studies suggest that citric acid has only a limited use. This includes neutralizing excess alkalinity in lye-peeled fruit or increasing the acidity of low acid fruit. Information on the possible protective effect exerted by citric acid upon the ascorbic acid retention in frozen packed fruits is extremely meagre. Data that have been published indicate that citric acid has very slight if any protective effect on ascorbic acid in frozen packed fruit and may even increase the loss of ascorbic acid if the product is held defrosted [Dubois *(3)*, Hohl, Buck and Rosoff *(7)*].

Tarr *(17)* and Tarr and Cooke *(18)* recently reported that dihydroxymaleic acid was as effective as ascorbic acid in preventing browning in cut fruits although it was an ineffective antioxidant for fish. Tarr *(17)* estimated that dihydroxymaleic acid could be prepared for approximately one-fifth of the cost of ascorbic acid and that it appeared to be non-toxic.

Only limited data have been accumulated to date on the stability of ascorbic acid in frozen packed fruit [Dubois and Colvin *(4)*, Bauernfeind et al. *(2)*, Hohl and Swanburg *(8)*]. Bauernfeind et al. *(2)* found in general that after 8 to 10 months' storage, about 80 percent or more of the added crystalline ascorbic acid was retained in the freshly thawed fruit as biologically active (total) vitamin C while at least 50 percent and frequently 70 to 80 percent was retained as reduced ascorbic acid. Such information is necessary if vitamin claims are to be made. It is desirable that the packer have some idea of the quantity of ascorbic acid required for various fruits to take care of losses during time from preparation to serving. Bauernfeind et al. *(2)* have reported on the stability of ascorbic acid in defrosted packages held for various periods and on the effect of method of defrosting. There is little information available on the reduced and total ascorbic acid which may be present in fruit, or fruit and syrup exposed for various periods in a dish after defrosting.

The purpose of this report is to present the findings of a comparative study of the antioxidant values of the addition of varying quantities of ascorbic acid alone, ascorbic acid-citric acid mixtures, and of dihydroxymaleic acid alone in frozen packed fruits. Results of ascorbic acid-stability studies on freshly defrosted products and after definite exposure periods at room temperature in flat enamel dishes also are presented.

Experimental Procedure

Sucrose syrup packs were used in these studies for comparing antioxidant values and stability. Apricots

[a] Presented before the Ninth Annual I. F. T. Convention, San Francisco, Calif., July 12, 1949.

and peaches were packed in 45 percent syrup in 16 fluid ounce moisture-proof cylindrical cartons. Except in a few special tests, a 30 percent syrup was used for Italian prunes and a 35 percent syrup for sweet cherries. Unless otherwise noted, 10 ounces (284 grams) of fruit and 5 ounces (142 grams) of syrup were weighed into each package, making a total weight of 15 ounces (426 grams). Crumpled pieces of wax paper were placed on top of the packed fruit to assist in keeping the fruit submerged. The antioxidants were accurately weighed and added directly to each packed carton, the latter then being inverted to mix the contents. During part of the study, the chemical agents were dissolved in the syrup just prior to packaging. Six or more replicates were packed for each test, together with a set of controls which contained no added antioxidant. A number of the experiments were repeated over a three-year period.

Varying amounts of the pure crystalline forms of ascorbic acid alone, laboratory preparations of ascorbic acid-citric acid mixtures, a commercial preparation of ascorbic-citric acid mixture, and citric acid alone were employed on apricots, fresh prunes and sweet cherries with most intensive studies being made on a number of varieties of peaches. Dihydroxymaleic acid studies were made using two varieties of peaches; Valiant which has moderate browning tendencies, and Elberta which discolors rapidly. Dr. H. L. A. Tarr of the Pacific Fisheries Experimental Station, Vancouver, B. C., very kindly supplied the writers with the dihydroxymaleic acid for these experiments.

With the exception of prunes, several varieties of each fruit were employed. Apricots were prepared by halving and pitting. Peaches were halved, pitted, steam peeled and sectored (sliced). Prunes were halved and pitted. Sweet cherries were packed both pitted and unpitted. Operations were carried out quickly to prevent initial browning. Freezing was under static conditions at —4° F. (—20° C.) during the first two years' experiments, and the third year an air blast freezer at —20° F. (—29° C.) was used. The frozen products were stored at —4° F. Examinations and analyses were made after 8 to 10 months' storage except in one test where peaches were stored 16 months to determine what effect extended frozen storage had on ascorbic acid loss.

For examination, the packages were defrosted overnight in a refrigerator at 34 to 36° F. (1 to 2° C.) or at room temperature (68° F.) for about 6 hours. There were a few ice crystals still present when the packages were opened and the contents placed in flat white enameled pans for observation. After one-half hour exposure the temperature of the product ranged from 40 to 45° F. (4.5 to 7° C.); after one hour, 50 to 55° F. (10 to 13° C.), and after one and one-half to two hours, 55 to 60° F. (13 to 15.5° C.)

Method of Analysis. Packages of the frozen fruit were thawed over-night (approximately 16 hours) at 34 to 36° F. (1 to 2° C.) A few ice crystals still were present. The entire contents of the package were given a 5 to 10 second blend in a Waring blendor. Fifty-gram samples were taken and blended with 350 milliliters (ml.) of 0.4 percent oxalic acid and filtered. Aliquots were taken for determination of reduced ascorbic acid and also for dehydro-ascorbic acid when the latter was to be estimated. Dehydro-ascorbic acid was reduced with H_2S similarly to the procedure of Rubin, Jahns and Bauernfeind *(15)*. These investigators have pointed out the importance of estimating this biologically active, reversibly-oxidized form of ascorbic acid in frozen packed fruits. Preliminary tests did not indicate any significant formation of interfering substances by the H_2S treatment on the products under examination. Ascorbic acid in peach samples was determined by visual titration with indophenol dye using a 5 to 10 second end point. On the highly colored extracts obtained from prunes and cherries, the determination was carried out colorimetrically in a Klett-Summerson photoelectric colorimeter using essentially the method of Loeffler and Ponting *(12)*. A standard curve was used instead of a constant. Several replicates were analyzed in every case.

Comparison of Ascorbic Acid and Combination of Ascorbic and Citric Acids on Color and Flavor

Apricots and Peaches. With apricots and peaches, ascorbic acid was added to each 15 ounces finished pack alone and in combination with citric acid in the following amounts: 100, 150, 175, 200 and 250 milligrams (mg.). Citric acid was added similarly at the rate of 355 and 637 mg. per package. A commercial ascorbic-citric acid combination also was applied to some peaches according to the suggested rate of 6 grams per 15 ounce package. Our analyses showed this product to contain 4.5 percent ascorbic acid and 95.5 percent citric acid. The most satisfactory results were obtained in these fruits with 150 to 200 mg. of ascorbic acid alone. One hundred fifty milligrams of ascorbic acid appeared to be the minimum quantity necessary to obtain reasonably beneficial effects with most varieties, in retaining flavor and color of thawed fruit for at least one hour's exposure in a flat dish. With rapid-browning varieties, such as the Elberta peach, 200 mg. is more satisfactory. Generally, amounts greater than 200 mg. appear to be of little advantage. These large quantities, while preventing discoloration for a long period, do not prevent deterioration in quality in other respects, especially in texture, and they tend to have not only a bleaching effect on the color, but also a reducing effect on the full flavor of peaches. Among the peach varieties being tested there were several non or slowly browning varieties. While it was not essential to add ascorbic acid to these, it was considered that the addition of 150 mg. of ascorbic acid improved the product.

Citric acid at the rate of 355 and 637 mg. per 15 ounces of fruit and syrup (that is, citric acid made up 0.08 to 0.15 percent of the weight of fruit and syrup or 0.25 to 0.45 percent of the weight of the syrup only) was of no benefit with respect to prevention of browning and it affected undesirably the flavor, particularly of peaches. At these concentrations it produced in peaches a sharp and somewhat bitter flavored product. This characteristic was most apparent in less sweet tasting varieties such as Elberta and J. H. Hale. The poorest samples both in flavor and color of frozen packed peaches were obtained with the commercial mixture of ascorbic and

citric acid. This mixture not only gave inadequate antioxidant control but made the peaches practically inedible. Citric acid added at the rate of 355 mg. per 15 ounces of finished pack lowered the pH of the syrup in the defrosted product approximately 0.2 pH, twice that amount about 0.35 pH, while the commercial preparation reduced the pH by 0.6. The citric acid affected the color of the syrup, making it slightly pinkish, the intensity of color increasing with increasing amounts of citric acid. Ascorbic acid alone in quantities up to 250 mg. did not significantly affect the pH value.

The amounts of ascorbic acid found to be effective in preventing browning in apricots and peaches, namely 150 to 200 mg. per 15 ounces, is in general agreement with reports in the literature *(6, 11, 14, 20)*. The results of this study also indicate that citric acid, when employed in amounts permitting an edible product, has no antioxidant value. This is in agreement with the findings of Dubois *(3)*. Consequently any advantage to be derived from citric acid would be in increasing the acid content of low acid fruits to enhance the flavor.

Italian Prunes. Fresh Italian prunes were frozen packed with the following weights of ascorbic acid being added per 15 ounces of product: 100, 150, 200, 250 and 300 mg. The addition of ascorbic acid proved to have but little beneficial effect. Prunes were found to make a relatively unsatisfactory dessert pack, the general opinion being that the canned product was much superior. Frozen packed prunes were preferred stewed or made into pies. It may be of interest to report that in a concurrent study on methods of preserving fresh prunes by freezing, display lugs or crates (3½ by 10 by 16½ inches) were packed full of whole prunes and frozen at —4° F. On examination after 8 months' storage, these prunes were found satisfactory for restaurant use.

Sweet Cherries. The first studies conducted in this laboratory on cherries revealed that red sour cherry varieties made quite acceptable products, but that sweet cherry varieties resulted in a relatively poor frozen product. Winter *(20)* recently reported that pitted sweet cherries packed with 150 to 175 mg. of ascorbic acid per pound of fruit and syrup plus a small amount of citric acid resulted in an acceptable frozen product. In further studies at this laboratory on sweet cherries the best frozen packs were obtained by using 355 to 637 mg. of citric acid and 175 to 250 mg. of ascorbic acid per 15 ounces of fruit and syrup, which is in general agreement with the recommendations of Winter *(20)*. The citric acid markedly enhanced the flavor and color. Royal Ann, Bing and Lambert were the varieties used. Cooking or stewing improved the acceptability of the fruit to most tasters, with improvement being most marked with the black varieties. All lots of cherries packed unpitted (whole) were definitely unsatisfactory having a very undesirable oxidized flavor.

Dihydroxymaleic Acid as a Possible Fruit Antioxidant

Ascorbic acid and dihydroxymaleic acid were employed each at the rate of 150 and 200 mg. per 15 ounces of product. Two varieties of freestone peaches, Valiant and Elberta, were used in this comparative study. Ascorbic acid and dihydroxymaleic acid were found to be equally effective when employed at the same rate as antioxidants for the Valiant variety which has a moderately active oxidizing enzyme system. However, with Elberta peaches, which are a very rapidly browning variety, ascorbic acid was found to be superior in preventing discoloration. For instance, after one-half hour exposure in a flat dish at room temperature, both ascorbic acid and dihydroxymaleic acid treated Elberta samples were satisfactory, but after one hour only the ascorbic acid lots were passable. There was no detectable difference in flavor between ascorbic acid and dihydroxymaleic acid treated fruit.

Some difficulty was experienced in dissolving dihydroxymaleic acid in the syrup. When dissolved in a water or sugar solution at room temperature, dihydroxymaleic acid was found to be relatively unstable under neutral or acid conditions and in the dark. Dihydroxymaleic acid has the further disadvantage that it interferes with the indophenol dye method of estimating ascorbic acid.

The results indicate that dihydroxymaleic acid is not as satisfactory an antioxidant for fruits as l-ascorbic acid. Since ascorbic acid is a natural accessory food factor and has become fairly inexpensive, it should be used in preference to an unnatural agent even if the latter is less costly and is quite non-toxic.

Stability of Ascorbic Acid in Frozen Packed Fruit

Table 1 summarizes three years' data on the effect of the addition of various quantities of citric and ascorbic acids on the reduced ascorbic acid content of syrup packed frozen peaches when defrosted after 8 months' storage at —4° F. Samples were analyzed immediately following thawing. The small quantity of ascorbic acid naturally present in the fruit is included in the calculation of degree of retention of ascorbic acid.

TABLE 1

Percent Retention of l-Ascorbic Acid in Peaches Frozen with Different Amounts of Ascorbic or Citric Acids and Analyzed After 8 Months' Storage at —4° F. (—20° C.)

Variety of peach	Citric acid added per 15 oz.	Percent retention of ascorbic acid					
		Ascorbic acid added per 15 oz.					
		0 mg.	100 mg.	150 mg.	175 mg.	200 mg.	250 mg.
	mg.	%	%	%	%	%	%
Elberta	0	21	57	70	....	76	77
	355	18	69	64	....	81	72
	637	19	71	80	....	79	....
J. H. Hale	0	26	64	69	....	78	83
	355	19	....	60	....	....	72
Valiant	0	42	75	78	79	91	85
	355	27	....	68	....	....	79
Vedette	0	33	70	78	89	79	83
	355	27	82	83	....	88	80
	637	....	81	82	....	85	....
Veteran	0	38	79	77	76	90	85
	355	22	....	75	....	....	81
Golden Jubilee	0	32	....	83	....	85	....
Redhaven	0	38	....	....	80	....	....
Rochester	0	62	....	81	....	....	....
Spotlight	0	29	....	87	84	87	88
Vanguard	0	43	....	88	....	93	86
V250810	0	35	....	84	....	....	....
V-Freeze	0	35	....	81	....	....	....

There was generally good agreement between the three years' results. It may be noted from Table 1 that retention of the originally added ascorbic acid always exceeded 50 percent l-ascorbic acid and usually was of the order of 80 percent. A few lots retained more than 90 percent of the added ascorbic acid in the reduced form. Elberta and J. H. Hale varieties tended to show a slightly greater loss of ascorbic acid than the other varieties although frequently there was no significant difference. With the exception of Elberta and J. H. Hale peaches the smaller amounts of added ascorbic acid showed the same degree of retention as the larger quantities. Also, with the possible exception of the minimum amounts of ascorbic acid, the addition of citric acid in amounts which still permitted an edible product had no significant beneficial effect on the retention of l-ascorbic acid in peaches.

Studies on prunes (Table 2) and sweet cherries (Table 3) yielded similar results. Italian prunes retained 58 to 81 percent of the added ascorbic acid in the reduced form. Retention of l-ascorbic acid in pitted sweet cherries was poorer and more variable than in unpitted cherries or in other fruits. It ranged from 36 to 54 percent for pitted and 65 to 78 percent for unpitted cherries.

TABLE 2

Percent Retention of l-Ascorbic Acid in Italian Prunes Frozen with Different Amounts of Ascorbic Acid and Analyzed After 8 Months' Storage at —4° F. (—20° C.)

Ascorbic acid added per 15 oz.	Percent ascorbic acid retained
mg.	%
0	0
100	66
150	81
200	73
250	77

Tables 3 and 4 present information on the relative amounts of total ascorbic acid (reduced and dehydroascorbic), ascorbic acid (reduced) and dehydroascorbic acid in frozen sliced peaches and pitted cherries after 8 to 10 months' storage. From 89 to 96 percent of the total ascorbic acid originally added was retained in peaches. Of this, dehydroascorbic acid accounted for

TABLE 3

Influence of the Addition of Ascorbic and Citric Acids on the Total, Reduced and Dehydroascorbic Acid Content of Pitted Sweet Cherries After 10 Months' Storage at —4° F. (—20° C.)

Variety of cherry	Treatment		Ascorbic acid content as packed (original)[b]	Ascorbic acid content after storage			
	Ascorbic acid added per package	Citric acid added per package		Total ascorbic acid	Total ascorbic acid as % of original[c]	Reduced ascorbic acid as % of original	Dehydroascorbic acid as % of total ascorbic acid
	mg. per 426 g.	*mg. per 426 g.*	*mg. per 100 g.*	*mg. per 100 g.*	%	%	%
Lambert	0	0	7.9	5.7	72	0	100
	100	355	31.3	27.8	89	42	53
	175	0	48.9	38.8	79	40	50
	175	355	48.9	38.3	78	45	42
	175	637	48.9	36.5	75	36	52
	175	850	48.9	41.1	84	54	35
	250	355	66.5	59.2	89	50	44
Bing	0	0	3.1			39	
	175	0	44.1			47	
	175	355	44.1			44	
	175	637	44.1			52	
Royal Ann	0	0	9.1			0	
	175	0	50.2			41	
	175	355	50.2			47	
	175	637	50.2			43	

[b] Includes average value for the presence of naturally occurring ascorbic acid.
[c] Percent figures calculated to nearest whole number.

TABLE 4

Influence of the Addition of Ascorbic Acid on the Total, Reduced and Dehydroascorbic Acid Content of Peaches After 8 Months' Storage at —4° F. (—20° C.)

Variety of peach	Treatment	Ascorbic acid content as packed (original)[d]	Ascorbic acid content after storage			
	Ascorbic acid added per package		Total ascorbic acid	Total ascorbic acid as % of original[e]	Reduced ascorbic acid as % of original	Dehydroascorbic acid as % of total ascorbic acid
	mg. per 426 g.	*mg. per 100 g.*	*mg. per 100 g.*	%	%	%
Elberta	0	3.7	3.7	100	38	62
	150	38.9	37.0	95	78	18
	200	50.6	46.3	91	76	17
Valiant	0	3.7	3.9	100	49	54
	150	38.9	34.6	89	81	10
	175	44.8	42.9	96	79	18
Vedette	175	44.8	43.1	96	89	7
Veteran	175	44.8	41.4	92	76	18
Spotlight	0	3.7	2.8	76	22	71
	175	44.8	42.2	94	84	11
Redhaven	0	3.7	3.8	100	38	63
	175	44.8	41.9	94	80	14

[d] Includes average value for the presence of naturally occurring ascorbic acid.
[e] Percent figures calculated to nearest whole number.

7 to 18 percent. Pitted cherries showed a total ascorbic acid retention of 75 to 89 percent of their original content with dehydroascorbic acid comprising 35 to 52 percent. Unpitted cherries showed no loss of total ascorbic acid with dehydroascorbic making up from 25 to 31 percent of the total ascorbic acid content. In control samples containing only naturally present ascorbic acid, the dehydroascorbic acid formed from 54 to 73 percent of the total value in peaches and 100 percent in the case of cherries. While results were not consistent, it appeared that citric acid may have had a slight protective effect on the added ascorbic acid in sweet cherries.

The findings of this investigation on the stability of ascorbic acid in frozen fruit processed with crystalline ascorbic acid in general substantiate those reported by Bauernfeind et al. *(2)*.

Effect of Length of Storage on Retention of Ascorbic Acid

In Table 5 the results are presented of a study on the effect of length of storage on the retention of added ascorbic acid in frozen packed peaches. The data show that there was no further loss of ascorbic acid during the 8 to 16 months' storage period. This is in agreement

TABLE 5

Effect of Length of Storage at —4° F. (—20° C.) on the Retention of Ascorbic Acid in Frozen Peaches Containing Different Amounts of Added Ascorbic or Citric Acids

Variety of peach	Treatment		% Retention of ascorbic acid after	
	Ascorbic acid added per package	Citric acid added per package	8 months	16 months
	mg./426 g.	*mg./426 g.*	%	%
Elberta	150	0	59	59
	150	355	51	60
	250	0	72	77
	250	355	72	73
J. H. Hale	150	0	58	60
	150	355	60	59
	250	0	79	77
	250	355	72	76
Valiant	150	0	72	....
	150	355	68	67
	250	0	76	80
	250	355	79	80
Vedette	150	0	79	72
	150	355	78	74
	250	0	80	91
	250	355	80	89
Veteran	150	0	75	73
	150	355	75	71
	250	0	83	79
	250	355	81	81
Rochester	150	0	81	81
Spotlight	250	0	88	86

with the observation of Joslyn *(10)* on the retention of naturally present ascorbic acid that there was no increase in ascorbic acid loss with increase in length of storage period. It also may be noted that citric acid used at the rate of 355 mg. per 426 grams of finished product had no effect on the ascorbic acid content.

Stability of Ascorbic Acid in Served Sliced Peaches

The effect on ascorbic acid retention of exposure of sliced peaches and syrup, and sliced alone, in flat dishes at 68° F. (20° C.) is shown in Tables 6 and 7. It is apparent from this data that total ascorbic acid remained relatively constant even after a period of 2 hours, although there was some loss in the 2-hour sample of fruits alone. With increased exposure time, l-ascorbic acid decreased and dehydroascorbic acid increased so that at the end of 2 hours the percentage of dehydroascorbic acid present had increased two-fold. However, there was still about 70 percent of the ascorbic acid present in the reduced form. The more enzyme-active

TABLE 6

Effect of Exposure in a Flat Dish on the Ascorbic Acid Content of the Combined Fruit and Syrup of Defrosted Sliced Peaches

Variety of peach	Exposure time	Ascorbic acid content of fruit and syrup		
		Total ascorbic acid	Reduced ascorbic acid as % of total	Dehydroascorbic acid as % of total
	hrs.	*mg./100 g.*	%	%
Elberta	0	46.3	83	17
	1½	45.8	65	35
Valiant	0	42.9	82	18
	1	42.5	76	24
	1½	42.9	80	20
	2	42.5	70	30
Spotlight	0	42.2	89	11
	1	41.5	87	13
	2	39.8	75	25

TABLE 7

Effect of Exposure in a Flat Dish on the Ascorbic Acid Content of the Fruit Only of Defrosted Sliced Peaches

Variety of peach	Exposure time	Ascorbic acid content of fruit		
		Total ascorbic acid	Reduced ascorbic acid as % of total	Dehydroascorbic acid as % of total
	hrs.	*mg./100 g.*	%	%
Valiant	0	35.6	64	36
	1	35.2	58	42
	1½	35.2	51	49
	2	30.8	38	62

peach variety, Elberta, showed greater loss of reduced ascorbic acid than Valiant, and Valiant in turn slightly greater loss than Spotlight, the variety with the least browning tendency. Changes in the exposed fruit alone were similar to those found for fruit and syrup but much more marked. These data indicate that the served product can be unacceptable in color and flavor and still retain most of the added crystalline ascorbic acid as biologically active vitamin C.

Summary

A report is given on a comparative study of the antioxidant value in frozen fruit of crystalline ascorbic, citric, and dihydroxymaleic acids.

Citric acid proved to be of no significant value in preventing oxidation or in reducing the quantity of ascorbic acid required when used in amounts permitting an edible product. Citric acid adversely affected the flavor of apricots and peaches but when employed with sweet cherries at the rate of 355 to 637 mg. per 15 ounces of finished product it enhanced the color and flavor of this fruit.

Dihydroxymaleic acid was not as satisfactory an antibrowning agent as ascorbic acid. Ascorbic acid added at the rate of 150 to 200 mg. per 15 ounces of fruit and syrup gave satisfactory results with apricots, peaches and sweet cherries.

Analyses revealed that the defrosted product after 8 to 16 months' frozen storage still retained normally 80

percent or more of the added crystalline ascorbic acid as total ascorbic acid (biologically active vitamin C). Fifty to 80 percent of the ascorbic acid remained in the reduced form. Loss of ascorbic acid did not increase with length of storage. The served product, even when starting to become unattractive in appearance, still retained most of the added ascorbic acid as biologically active vitamin C with about 70 percent of it present in the reduced form.

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Antioxidants in the Hemoglobin Catalyzed Oxidation of Unsaturated Fats[a, b]

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Phenolic inhibitors retarded the hemoglobin catalyzed oxidation of lard. Synergistic antioxidants, with the exception of ascorbic acid, had no effect in the presence of hemoglobin except at very low concentrations or after coagulation of the hemoglobin. Ascorbic acid markedly retarded rancidity in the presence of hemoglobin or nitrosohemoglobin and small amounts of tocopherol. It caused fading and greening of hemoglobin but protected the red color of nitrosohemoglobin solutions and cured meat. Possibilities for its use in cured meats are discussed.

The catalytic effect of hemoglobin and other iron porphyrins on the oxidation of unsaturated fats was first demonstrated by Robinson *(9)* and has been further studied by a number of investigators. Peroxidation of unsaturated fatty acids by hemins is accompanied by destruction of the hemin molecule with loss of color *(4)*. This reaction is believed to be an important factor in the discoloration and development of rancidity in meats *(13)*.

The experiments here reported were designed to test the effectiveness of various antioxidants in retarding the hemoglobin-unsaturated fat reaction, with the ultimate objective of selecting antioxidants which might be suitable for retarding oxidative changes in meats.

Banks *(1)* studied a variety of antioxidants in suspensions of linolic acid in buffered starch solutions containing hematin as a catalyst. He found the ortho and para hydroxy phenols to have a pronounced antioxidant effect, but various other substances tried, including phosphates, citric acid, sulfites, nitrites, sorbitol, etc., were without protective action. The same substances were not tried in the absence of the hematin.

Recent studies in this laboratory have shown that synergistic antioxidants may behave very differently in aqueous fat as compared to dry fat systems *(10, 12)*. Of a number of synergists tried, only citrate and polyphos-

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phates significantly inhibited rancidity when the fat was in contact with water. These compounds were therefore selected for study in the hemoglobin catalyzed reaction. Further studies were also carried out on ascorbic acid, since it had been shown that ascorbic acid retarded rancidity in hemoglobin catalyzed oxidations provided the fat contained sufficient phenolic inhibitors. Antioxidant indices obtained with ascorbic acid in the presence of hemoglobin were higher than those obtained without the hemoglobin.

Methods

The method of preparing hemoglobin solutions and muscle extracts and of measuring the hemoglobin content of such preparations has been previously described *(14)*.

For preparation of nitrosohemoglobin, in most of the present experiments the directions of Jensen *(5)* were followed, i.e. sufficient sodium hydrosulfite was added to a hemoglobin solution to convert oxyhemoglobin and methemoglobin to reduced hemoglobin as evidenced by change in color from red to the deeper purplish red of venous blood. The reduced hemoglobin was then converted to nitrosohemoglobin by the addition of sufficient sodium nitrite to obtain the characteristic red color of the cured meat pigment. For solutions containing approximately .4% hemoglobin, about 24 mgs. of sodium hydrosulfite and 2 ml. of a 1% solution of sodium nitrite were needed for 100 ml. hemoglobin solution.

When such preparations are to be used to determine the effect of nitrosohemoglobin on rancidity, it is important to avoid a great excess of either sodium hydrosulfite or sodium nitrite, since both of these reagents had a definite accelerating effect on rancidity in lard. In an attempt to avoid the use of hydrosulfite, oxyhemoglobin solutions were reduced by passing hydrogen through them. However, formation of the nitrosohemoglobin seemed less complete after reduction by this method. The pigment obtained on the addition of sodium nitrite faded very rapidly. The use of ascorbic acid as a reducing agent is described under results.

The procedures used for obtaining contact between the aqueous solutions and the fat and for measuring time required for the fat to turn rancid have been described fully elsewhere *(10, 12)*.

Results

Phenolic inhibitors. Lard containing various phenolic inhibitors was brought into contact with hemoglobin solutions previously adjusted to pH 5.6 by the addition of HCl. These inhibitors, at concentrations of .005% of the weight of lard, had approximately the same order of effectiveness in the presence of hemoglobin as in its absence (Table 1). Nordihydroguiaretic Acid (NDGA) was markedly superior to either tocopherol or propyl gallate under the conditions of this experiment.

Synergistic antioxidants. The activity of synergistic antioxidants in aqueous fat systems containing hemoglobin did not parallel their activity in the absence of the hemoglobin.

Within the pH range of normal meat as well as at higher pH values citrate and polyphosphates were shown to have marked antioxidant properties in artificial aqueous fat systems *(12)*. The two most effective of this group of compounds were insoluble sodium metaphosphate or Maddrell's Salt $(NaPO_3)x$ and the product known commercially as "hexametaphosphate," a soluble, polymerized sodium metaphosphate also of indefinite molecular weight.

TABLE 1

Effects of Phenolic Inhibitors on Hemoglobin Catalyzed Oxidation of Lard (pH 5.6)

Added Inhibitor	Time to Turn Rancid		
	.2% Hb 25° C.	No Hb 25° C.	No Hb 45° C.
	Hours	*Days*	*Days*
None	7	12	3
Tocopherol (.005%)	11	45	6
Propyl gallate (.005%)	30	45	..
N D G A (.005%)	>47	>70	26

These two metaphosphates and citric acid were adjusted to the pH of a muscle extract (5.6) and their effect on rancidity of plain lard measured both in the presence and absence of muscle extract (Table 2). The

TABLE 2

Effect of Citrate and Polyphosphates on Rancidity in Presence of Muscle Extract

(Substrate = plain lard; pH adjusted to 5.6, Hb concentration of muscle extract = .1%)

Synergist (0.1%)	Time to Turn Rancid	
	No Muscle Extract	Muscle Extract
	Days	*Hours*
Control	2	8-10
Citrate	4	8-10
"Hexametaphosphate" $(NaPO_3)_n$	13	6
Maddrell's Salt $(NaPO_3)x$	13	8-10

concentrations of hemoglobin and synergists in this and subsequent tables represent final concentrations of the solutions in contact with the fat. Equal volumes of solution and fat (1.5 ml.) were used throughout. While citrate doubled the keeping time of the fat and the metaphosphates increased keeping time 6 to 7 times in the absence of the muscle extract, none of these antioxidants protected in the presence of the extract. "Hexametaphosphate" caused a precipitation of protein in the muscle extract and a slight acceleration of rancidity.

A similar lack of protection resulted when hemoglobin at approximately the same concentration was substituted for muscle extract and when .005% tocopherol was added to the lard. Other polyphosphates, including sodium heptaphosphate ($Na_9P_7O_{22}$), sodium triphosphate ($Na_5P_3O_{10}$) and pyrophosphate were also ineffective in the presence of either hemoglobin or muscle extract. All of these phosphates inhibited rancidity when hemoglobin was not present. The heptaphosphate, like "hexametaphosphate" caused a precipitation of protein, whereas no precipitation occurred with the triphosphate or pyrophosphate. Orthophosphates and a number of amino acids were also tried. These were ineffective in aqueous fat systems, however, either with or without hemoglobin.

Since in all of the above trials of synergistic antioxidants, with hemoglobin, rancidity developed within a few hours under the conditions of the test, it was

thought possible that there was insufficient time for the synergist to enter the fat. Accordingly the tocopherol content of the lard was increased sufficiently so that several days were required for the control sample to turn rancid. Even under these conditions, with the exception of ascorbic acid, no synergist tried had any retarding effect on the development of rancidity in the presence of normal concentrations of unheated muscle extract (Table 3). It was possible to demonstrate a protective effect with very low hemoglobin concentrations (Table 4).

TABLE 3

Effect of Synergists on Rancidity in Presence of Muscle Extracts
(Substrate = lard + .02% tocopherol, Hb content of muscle extract = .1%; pH = 5.4)

Synergist (0.1%)	Days to Turn Rancid (H_2O = 22 days)	
	Unheated Muscle Extract	Heated, Unfiltered Muscle Extract
Control	4	23
Ascorbic Acid	>55	>55
Citrate	4	39
Pyrophosphate ($Na_4P_2O_7$)	4	43
Triphosphate ($Na_5P_3O_{10}$)	4	46

TABLE 4

Effect of Synergists on Rancidity in Very Dilute Hemoglobin Solutions
(Substrate = lard + .02% tocopherol; .02 molar acetate buffer pH 5.4; Hb = .04%)

Synergist (0.1%)	Days to Turn Rancid		
	Acetate Buffer No Hb	Unheated Hb	Heated and Filtered Hb
Control	28	10	31
Ascorbic Acid	26	23	27
Pyrophosphate ($Na_4P_2O_7$)	43	28	43
Triphosphate ($Na_5P_3O_{10}$)	50	27	43

Heated hemoglobin solutions and muscle extracts. Watts and Peng *(14)* found practically complete loss of catalytic activity in both hemoglobin solutions and muscle extracts on heating to a temperature of 90° C. On the other hand Reiser *(8)* obtained only slight diminution of the catalytic effect of hemoglobin solutions on heating to the boiling point and considered the relatively slight loss obtained to be due to inactivation of another lipoxidase rather than to any change in activity of the hemoglobin itself. The apparent discrepancies in these reports are probably traceable to differences in the conditions of heating, particularly the pH of the hemoglobin solutions. Reiser prepared his hemoglobin in M/10 K H_2 P O_4 (pH 4.7) and diluted with acetate buffers at pH 5.0 so that the actual pH at the time of heating was presumably between 4.7 and 5.0. In this pH range hemoglobin is far removed from its isoelectric point and relatively stable. As the pH is increased the hemoglobin becomes less stable and at pH values approaching the isoelectric point of 6.8 it is completely coagulated, leaving a clear colorless solution, after relatively mild heat treatment. While it is undoubtedly true, as Reiser has pointed out, that the active heme fraction is not readily destroyed by heat, it can be thrown out of solution by coagulation of the hemoglobin.

In the present experiments muscle extracts and hemoglobin solutions were heated in the pH range 5.4 to 5.6. Heating of muscle extracts to 90° C. at this pH invariably destroyed all catalytic activity; in fact such heated solutions usually inhibited rancidity slightly even when the coagulum was not filtered off (Table 3). Probably the presence of the readily coagulable muscle proteins aids in the complete precipitation of the hemoglobin. The activity of hemoglobin solutions prepared from laked red cells was always greatly reduced but usually not completely eliminated by heating to 90° C. at this pH. Differences in the rate of heating, in the time of standing after heating (since aggregation of the denatured protein continues slowly as the solution stands) and in buffer salts used all may effect the coagulation. If heated hemoglobin solutions which had not lost all of their activity were filtered, the activity was further reduced.

Citrate and several of the lower polyphosphates regularly showed about the same inhibiting effect on rancidity in hemoglobin or muscle extract preparations heated enough to destroy their catalytic effect as they did in the absence of hemoglobin (Tables 3 and 4). The higher polyphosphates were not tried.

Ascorbic acid and hemoglobin. Unlike the other synergists tried, ascorbic acid gave a striking degree of protection with hemoglobin solutions and muscle extracts when the fat contained .005% or more tocopherol (Tables 3 and 4). An earlier report from this laboratory *(10)* demonstrated that ascorbic acid rapidly accelerated the oxidation of plain lard in contact with water. Further experiments have given consistent acceleration with ascorbic acid when .005% tocopherol was added to the lard, and even at tocopherol levels of .02% ascorbic acid gave little or no protection in aqueous fat systems. In the presence of hemoglobin solutions or muscle extracts the antioxidant indices obtained with ascorbic acid were always higher than those obtained in the absence of hemoglobin. They ranged in the present study from 2 to values greater than 20. The lower values were obtained with smaller concentrations of hemoglobin.

However, ascorbic acid also had the effect of rapidly destroying the red color of hemoglobin solutions. The addition of .1% ascorbic acid to hemoglobin solutions at 45° C. caused noticeable dulling of the red color within an hour, followed by the gradual conversion of hemoglobin to a greenish pigment which eventually precipitated. In the absence of the ascorbic acid, oxidation of the hemoglobin was much slower and did not pass through a green stage.

Lemberg, et al. *(7)* demonstrated the formation of green bile pigments from hemoglobin in the presence of ascorbic acid. Hydrogen peroxide also readily produces green decomposition products from hemoglobin *(3)*. It is possible that hydrogen peroxide formed during the oxidation of ascorbic acid is responsible for the pronounced effect of the ascorbic acid on the oxidative decomposition of the hemoglobin.

Catalase is present, of course, in both hemoglobin preparations from laked blood cells and also in muscle extracts. Rapid evolution of gas followed the addition of .0057 molar hydrogen peroxide (equivalent to .1% ascorbic acid) to hemoglobin solutions. There was no

visible evolution of gas upon the addition of ascorbic acid. It is possible that, since the oxidation of ascorbic acid is catalyzed by the hemoglobin, hydrogen peroxide is formed in such close proximity to the hemoglobin that it can act upon the hemoglobin before being decomposed by any catalase present. In general, the rate of discoloration of a given hemoglobin solution was greater in the presence of ascorbic acid than in the presence of the same molar concentration of hydrogen peroxide, probably because of the decomposition of a large fraction of the hydrogen peroxide before it could act on the hemoglobin.

Ascorbic acid and nitrosohemoglobin. Nitrosohemoglobin, the red pigment of cured meat, prepared experimentally by the addition of nitrite to hemoglobin which had been reduced with hydrosulfite, catalyzed the oxidation of lard to the same extent as did the original hemoglobin solution. Ascorbic acid retarded the nitrosohemoglobin catalyzed oxidation of the fat. Hydrogen peroxide produced a greenish oxidation product with nitrosohemoglobin similar to that obtained with hemoglobin, except that the action of the hydrogen peroxide was much more rapid and drastic with the nitrosohemoglobin than with the original hemoglobin, probably because of the destruction of catalase during the conversion of hemoglobin to nitrosohemoglobin.

In sharp contrast with its dulling effect on the color of hemoglobin, however, ascorbic acid markedly protected the original bright red color of nitrosohemoglobin. In the absence of ascorbic acid, nitrosohemoglobin faded more rapidly than did hemoglobin upon exposure to air. The addition of .1% ascorbic acid to nitrosohemoglobin solutions greatly increased the time of normal color retention. When the color of such solutions did begin to fade, however, a green or grey green hemochromogen was formed, whereas in the absence of the ascorbic acid the decomposition products were usually brownish grey, not green. Too much emphasis should probably not be placed upon color of the final decomposition products, since these developed only after several days at 45° C. and could well be influenced by bacterial action.

Results of a typical experiment illustrating the points discussed above are given in Table 5. The hemoglobin and nitrosohemoglobin used in this experiment were aliquots of the same sample of laked blood, adjusted to pH 5.6 by the addition of HCl. The ascorbic acid solution was also adjusted to pH 5.6 by the addition of NaOH before adding to the hemoglobin. The concentrations of hemoglobin and ascorbic acid given in the table represent final concentrations after adjusting and mixing.

The rancidity tests were carried out as usual with the aqueous solutions on filter papers in contact with the carotene—lard. The hemoglobin did not impart sufficient color to the papers to make it possible to follow color changes of the solutions in contact with the fat. However, color changes were followed by observing aliquots of the same solutions in test tubes held at 45° C. No fat was present in the test tubes; fading of hemoglobin has been shown to occur very rapidly during the early stages of the oxidation of the fat *(13)*.

TABLE 5

Color Changes and Oxidase Activity of Hemoglobin and Nitrosohemoglobin

Aqueous Solution (Hb conc = .3%; pH = 5.6)	Hours to Turn Rancid (Substrate = lard + .005% tocopherol)	Color Changes in Absence of Fat
Hemoglobin	4	Bright red for 5 hrs.; dull after 20 hrs.
Nitrosohemoglobin	4	Faded slightly in first few hours; same as Hb after 20 hrs.
Hemoglobin + .0057M H_2O_2	4	Gas evolved; greenish brown in 3 hrs.
Nitrosohemoglobin + .0057M H_2O_2	20	No gas evolved; green immediately, yellow then colorless within 3 hrs.
Hemoglobin + Ascorbic Acid (.1%)	70	Green in 2 to 3 hrs.
Nitrosohemoglobin + Ascorbic Acid (.1%)	70	Bright red for 24 hrs.; superior in color to all others after first 4 to 5 hours. Turning green after 2 days.

In one experiment the concentration of nitrosohemoglobin was varied over the range .05 to .40% with the same concentration of ascorbic acid (.1%). Marked color protection was obtained with the ascorbic acid at all concentrations. The ascorbic acid also protected lard with .005% added tocopherol against rancidity at all levels of nitrosohemoglobin. The lowest antioxidant index, 9, was obtained with the lowest nitrosohemoglobin concentration. Antioxidant indices ranged from 15 to >20 at the higher concentrations.

With a fixed concentration of .2% nitrosohemoglobin, antioxidant indices of 4, 16 and 13 respectively were obtained for .01%, .1%, and 1% ascorbic acid solutions. During the first few hours, the 1% concentration of ascorbic acid gave a brighter red color to the nitrosohemoglobin solution, but the subsequent greening also occurred earlier.

In an effort to eliminate this greening, further experiments on color were carried out in which sodium hydrosulfite was omitted altogether in the preparation of nitrosohemoglobin. Ascorbic acid (.1%) and the usual amount of sodium nitrite were added together to oxyhemoglobin solutions (all solutions having been previously adjusted to pH 5.6). Under these conditions a brilliant red color developed with ascorbic acid and nitrite after an hour or two at 45° C. The color took longer to develop, but was brighter and far more permanent than that obtained with either hydrosulfite and nitrite or hydrosulfite, nitrite and ascorbic acid. The secondary greenish pigments did not develop except after prolonged holding at 45° C. when ascorbic acid was the only reducing substance present. Greying and greening of all other variations took place long before the ascorbic acid-nitrite tubes lost their color. Apparently the hydrosulfite is involved in the greenish decomposition of nitrosohemoglobin in the presence of ascorbic acid.

Effect of ascorbic acid on color of cured pork. In order to test the effect of ascorbic acid on color of nitrosohemoglobin as it actually exists in cured meat, several experiments were performed in which cured pork

products were heated or dipped in .1% solutions of ascorbic acid, previously adjusted to pH 5.6 with NaOH. Commercial canned comminuted pork products, packed with sodium nitrite, were convenient to use because of the uniformity of the individual slices. When such products were sliced and exposed to air the cut surfaces gradually lost their bright red color and turned an unattractive grey. The fading was hastened by cooking.

In a typical experiment, uniform slices of the canned product were heated in distilled water and in .1% ascorbic acid (pH 5.6) at 90° C. for a period of 20 minutes, then placed in Petri dishes in the refrigerator. Noticeable fading had occurred in the water cooked samples by the end of the heating period and after 3 days in the refrigerator these slices were a uniform putty color. Those heated in ascorbic acid, on the other hand, still retained much of their original pink color at the end of 2 weeks of storage. Some additional protection was obtained by cooking in 1% rather than .1% ascorbic acid. At a level of .01% the protection was less but still noticeable.

Soaking slices in ascorbic acid for a 20 minute period also gave some protection as compared to controls soaked in water. At the end of one week of storage the water soaked samples were badly faded but not completely grey. Those soaked in ascorbic acid were distinctly better but not as bright as the slices cooked in the ascorbic acid.

No greening has been observed with the use of ascorbic acid on cured meat products even after long periods of refrigeration.

Discussion

Ascorbic acid appears to be by far the most promising of any synergist tried for preventing both rancidity and color loss in cured meats. The explanation for the strong accelerating effect of ascorbic acid on rancidity in aqueous lard systems and for its strong antioxidant effect when hemochromogens and small amounts of tocoperol are added to the system is still obscure. The accelerating effect is not due to hydrogen peroxide; the addition of hydrogen peroxide slightly inhibited rancidity.

It would be of interest to follow the rate of loss of reduced ascorbic acid in these systems in the presence and absence of hemochromogens. The fact that oxidation of ascorbic acid is catalyzed by hemochromogens *(2)* suggests that its antioxidant effect in the presence of hemochromogens may be due to an oxidation product or to a combination of such an oxidized product with the hemochromogen itself.

The protective effect of ascorbic acid on the color of the cured meat pigment can undoubtedly be ascribed to its strong reducing action. The necessity for reducing conditions for proper color fixation in cured meats has been emphasized repeatedly *(6, 11)*. The nitrate and nitrite present must be reduced to nitric oxide and oxygen must be removed from the hemoglobin before the two can combine. The resulting nitrosohemoglobin is also highly susceptible to oxidation.

Whether or not ascorbic acid can be successfully used at any stage in the preparation or preservation of cured meats remains to be demonstrated. The fact that ascorbic acid destroys the color of hemoglobin although it improves the color of nitrosohemoglobin would seem to make it imperative that sufficient nitrite be present if ascorbic acid is used.

The ascorbic acid and nitrite can be added together in pure hemoglobin solutions with good results; it seems possible that they might successfully be added together in the curing brine. It would also be worth trying the addition of ascorbic acid to cured meats before canning to prevent surface fading when the can is opened and the meat exposed to air.

The ability of ascorbic acid to prevent rancidity in cured meats is dependent upon the presence of both nitrosohemoglobin (or hemoglobin) and tocopherol or other phenolic inhibitor. Phenolic inhibitors introduced during the smoke plus the tocopherol naturally present might be sufficient to allow the high antioxidant indices which can be obtained with ascorbic acid. Again actual trials under normal curing conditions are essential before any conclusions can be drawn.

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The Longevity of the Coliform Bacteria and Enterococci in Iced Crabmeat

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A study was made of the numbers of coliform bacteria and enterococci in fresh iced crabmeat during the storage life of the product. The coliforms tended to increase during iced storage while the numbers of enterococci remained unchanged.

The methods employed in the preparation of crabmeat make it a product of unusual interest to the bacteriologist and sanitarian. The crabs are often taken from polluted waters, but the steaming or boiling process which precedes the picking of the meat effectively destroys the coliform bacteria and most other organisms *(26)*. The coliform bacteria found in market crabmeat therefore are added during the preparation or storage of the product. The picking, sorting, and packaging operations involve such intimate handling that some contamination with coliform bacteria seems unavoidable, but their presence in large numbers is indicative of careless methods in the preparation or storage of the meat. Of much more sanitary significance, however, than the coliform group as a whole is the presence in seafoods of the fecal *Escherichia coli (5, 6, 14, 15, 16)*. The distinction between the fecal and nonfecal members of the coliform group, however, offers serious difficulties *(1, 2, 3, 7, 8, 12, 13, 18, 22, 24)*.

The recent development of satisfactory methods for the detection of fecal streptococci in water and foods has suggested the possibility of using this group of organisms, rather than *E. coli*, or in addition to *E. coli*, as an indicator of lack of proper sanitation in the handling of crabmeat *(4, 10)*. This group of bacteria, recognized for a half-century as significantly related to fecal pollution *(17, 19)*, may now be investigated by methods *(4, 9, 21, 27, 28)* no more intricate than those employed for coliforms, and satisfactory recognition of the streptococci requires less time than does the coliform test.

Using the new and improved methods for the detection of the enterococci, Ostrolenk, Kramer and Cleverdon *(10)* concluded that "enterococci in artificially contaminated soils and pecan meats and in normal feces appear to survive longer than *Escherichia coli* under identical conditions of storage."

Additional information is needed on the problem of growth or survival of indicator organisms in seafoods. The purpose of the present work was to determine the survival or growth of the enterococci and the coliform group in fresh crabmeat held under normal conditions of storage.

Methods

The crabmeat, obtained from a commercial packer in New Orleans, was packaged in the customary 1-lb. cans with perforated bottoms and snap-on covers. On arrival at the laboratory (usually a day after the meat was packed), samples were taken for determination of coliforms, enterococci and aerobic plate count. The cans of crabmeat were stored in cracked ice in a room held at 40-45° F. (4-7° C.), and the determinations were repeated at intervals until the meat was spoiled.

At the time of sampling, the crabmeat was mixed as thoroughly as possible with a sterile spoon, and 15 grams were weighed directly into a sterile 200 ml. widemouth bottle containing a quantity of broken glass. One hundred and thirty-five ml. of water were added, and the bottle was placed horizontally on a Kahn shaker and shaken at high speed for three minutes. Serial dilutions of the sample were prepared in the usual way, and duplicate plates were prepared from appropriate dilutions. The plates were poured with nutrient agar (Peptone 5g, beef extract 3g, agar 15g per liter) and incubated for 3 days at room temperature (22-28° C.).

For the determination of coliforms, lactose broth *(23)* was employed in the presumptive test, using 5 replicate tubes of each dilution. Confirmation was in brilliant green lactose bile (2%), and the completed test (on certain samples only) was as prescribed in "Standard Methods for the Examination of Water and Sewage" *(23)*.

The presence of enterococci was determined by the method of Winter and Sandholzer *(28)*, using 5 replicate tubes of each dilution in the presumptive test. Incubation was at 45° C. in a thermostatically controlled water bath. Confirmation, using the "slant-broth" method, consisted of the development of pinpoint colonies on the slant, sediment in the broth, negative catalase test, and streptococcal morphology by microscopic examination.

During the latter part of the work, two separate determinations were made on each lot of meat at every examination period.

Results

The aerobic plate counts on the crabmeat, at the time of arrival in the laboratory, were found to vary between 260,000 and 10 million, with 68% of the samples exceeding 1 million per gram (Table 1). In most instances, there was no significant increase in numbers during the first 5 days of storage; often the 3-day count was lower than the initial count. These results, considered to be possibly erratic, together with fluctuations in the coliform and enterococci counts, led to the use

TABLE 1
Numbers of Bacteria in Fresh Iced Crabmeat

Plate count per gram	Number of samples [a]	% of samples
Less than 500,000	5	10
Over 500,000, less than 1 million	11	22
1-4 million	24	48
5-9 million	7	14
10 million and over	3	6

[a] 50 samples examined.

of duplicate samples for all determinations. Two separate samples were taken from each batch of meat at each period of testing. Typical results of the plate counts obtained during the holding period are presented in Table 2. Spoilage usually was obvious in 9 to 13 days at which time the bacterial count was generally over 1 billion per gram.

Results typical of the trend in numbers of coliforms and enterococci are indicated in Table 2 . Of all the samples studied, about 80 percent had initially less than 20 coliforms per gram, and about 50 percent of the samples had less than 20 enterococci per gram. With both groups unacountable fluctuations occurred, even when averages of duplicate determinations were used. The discrepancies apparently are inherent in the methods employed. It was observed that while the coliforms increased in some samples during storage, in others there was a decrease in numbers. Likewise, the streptococci apparently increased slightly in some samples but decreased in others. In most instances, however, the coliforms increased while the enterococci either decreased or showed little change in numbers. There appeared to be no relationship between the numbers of coliforms and the enterococci. The samples with fewer coliforms sometimes had most enterococci, and vice versa.

TABLE 2

Changes in Plate Count, Coliforms, and Enterococci in Crabmeat During Iced Storage

Sample	Days Storage	Plate count/gram [b] (times 1000)	M P N per gram	
			Coliforms [b]	Enterococci [b]
39	1	266	4.7	10.0
	7	95,500	10.5	20.0
	10	1,660,000	0	3.2
	13	1,575,000	1.0	6.8
40	1	1,180	6.4	32.5
	7	56,000	4.5	20.0
	10	4,600,000	36.0	10.4
	13	3,850,000	66.5	0
42	1	627	14.4	2.0
	3	737	21.5	4.9
	5	4,600	78.5	4.3
	7	115,500	350.0	9.5
	9	647,500	445.0	14.0
43	1	1,622	7.9	1.5
	3	1,345	8.2	1.1
	5	1,400	4.3	0.4
	7	114,700	160.0	2.5
	9	347,500	133.0	1.4
44	1	884	14.4	1.8
	3	985	9.0	1.4
	5	2,350	22.0	3.3
	7	297,750	160.0	3.6
	9	2,297,000	380.0	1.4
45	1	5,690	0.98	95.5
	3	26,000	1.8	126.0
	7	2,740,000	29.0	88.5
	9	3,120,000	78.5	124.5

[b] Figures are averages of 2 determinations.

Additional evidence of the growth of coliforms in the iced crabmeat during storage is presented in Table 3. In most instances the coliforms increased many fold during the normal storage period of 9 to 13 days. In no instance, however, was there a significant increase in the number of enterococci. The population trends of the two groups of organisms are clearly indicated in the column of averages in Table 3, which indicate a 100-fold increase in coliforms and no significant change in the enterococci.

TABLE 3

The Effect of Storage on the Numbers of Coliforms and Enterococci in Iced Crabmeat

MPN per gram [c]

Sample	Coliforms		Enterococci	
	1-3 days	9-13 days	1-3 days	9-13 days
37	16.9	4.8	20	0
38	2.0	0	20	0
39	4.7	1	10	6.8
40	6.4	66.5	32.5	0
41	4.6	78.0	23	23
42	14.4	445	2	14
43	7.9	875	1.5	1.3
44	14.4	410	1.8	1.4
45	0.73	5000	3.1	2.5
46	.98	85.5	95.5	124
Average	7.3	696.6	20.9	17.3

[c] Each figure is the average of two determinations.

On some samples determinations of M P N of total coliforms and of *E. coli* were made. The results, presented in Table 4, indicate that *E. coli* may persist in crabmeat throughout the period of storage, but there was no evidence of marked increase in numbers. It was noted that of 446 isolates from the limited number of "completed" tests 25 percent were citrate negative, of which 64 percent were indol positive. Only 16 percent of the *Escherichia* isolates grew at 45° C.

TABLE 4

Relationship of E. coli to Total Coliforms in Crabmeat Stored at 36-41° F. (2-5° C.)

M P N per gram based on "completed test" [d]

Sample	1 day		5-7 days		9-11 days		13-15 days	
	Coliforms	E. coli	Coliforms	E. coli	Coliforms	E. coli	Coliforms	E. coli
43	14	6.1	1.05	0.2	3.6	1.8	..	..
44	0.51	0.1	1.8	1.1	3.25	0	..	..
A	0	0	5.1	0.9	0.1	0	2.3	0.2
B	0	0	10.3	4.7	3.4	1.0	..	..
C	..	..	3.4	2.0	5.9	5.9	5.6	0.3
D	0	0	17.0	7.3	11.0	4.3	..	..
E	0	0	1.2	1.0	11.6	1.7	47.6	0.6
F	0	0	21.5	0.4	25.0	1.7	1.4	1.0

[d] Each figure is the average of two determinations.

Conclusions

Coliform bacteria in iced crabmeat tended to increase during the storage period, while the numbers of enterococci remained practically unchanged. *E. coli*, as indicated in a limited number of tests, persisted throughout the storage period, but did not increase significantly.

There apparently was no relationship between the initial total count and the numbers of coliforms or enterococci, nor any relationship between numbers of coliforms and enterococci.

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Relation of the Spray Residue Problem to Food Processing*

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(REVIEW PAPER)

The relation of the entomologist, the toxicologist, the analytical chemist and the food technologist to the spray residue problem is discussed and progress in the above fields is noted.

The responsibility of the insecticide manufacturers and dealers, the extension personnel, the farm advisors, and the field representatives of the food processors in obtaining and disseminating knowledge regarding spray residues is discussed.

Suggestions are given for conducting residue studies on raw and processed foods and precautions to be observed in the interpretation of results are discussed. Results obtained in recent studies of spray residues on tomatoes and apricots are summarized.

The problem of insecticide residues on food products has been with us for a considerable number of years; however, with the advent of DDT and the host of other new insecticides which have recently appeared on the market the problem has become of much greater concern to all food processors. It will be the purpose of the present paper to outline some of the problems involved in connection with insecticide residues as related to food processing and to give an appraisal of some of the progress made to date.

The subject under consideration covers a wide field of specialized skills and further progress will require the combined talents of a number of investigators of quite diversified training. In the following discussion the contributions which the entomologist, the toxicologist, the food technologist, and the analytical chemist can make to the solution of various residue problems confronting the food processor will be considered, and the relationship of the insecticide manufacturers and dealers, the extension personnel, the farm advisors, and the field representatives of the food processors to the spray residue problem will be discussed.

The Entomologist and the Spray Residue Problem

First of all, it is the province of the entomologist to determine which insecticides are of the greatest value in each particular set of circumstances, and to work out the best methods of application. In this regard the entomologist must take into consideration not only the

* Certain of the material in this paper was presented at the December 2, 1948 meeting of the Northern California Section of the Institute of Food Technologists at Berkeley, California.

conditions best suited to the control of insects, but also the procedures which will result in the minimum hazard from the residue standpoint. The primary concern of the entomologist, therefore, is to determine the most efficient and economical method of insect control which at the same time will not contaminate the raw product at the time of harvest with objectionable amounts of residue. Factors to be considered in this regard are: the most suitable insecticides, the best methods and times of application, the minimum amount of insecticide required to do the job, and the minimum length of time which should elapse between the last application of insecticide and the time of harvest.

The most efficient and economical methods of insect control must necessarily be determined by research entomologists by means of carefully controlled laboratory and field experiments, which must be continued over a period of several years in order to reach a complete solution. As soon as the research data justify modifying existing control recommendations, the research and extension entomologists in cooperation with specialists working with particular fruit and vegetable crops should bring up to date the official state recommendations for the control of insects on food crops. These recommendations then are given by both the entomology and horticulture staffs of the state agricultural extension service to the farm advisers and the field representatives of food processors who, in turn, disseminate these official state recommendations to growers. The field representatives of food processors, therefore, should be familiar with the official recommendations made each year for insect control methods on food crops, and they should also be kept informed of the control measures actually being used by the growers. The food technologist must depend on the field representatives of his company for the knowledge of what insecticides have been used and how they have been applied. This knowledge should be extended to include each field and each lot of raw product utilized by the processor. Insecticide dealers should also keep informed of these official recommendations and should be prepared to pass this information on to their customers.

The Toxicologist and the Spray Residue Problem

The toxicologist is often said to hold the key to the whole residue problem since the toxicity of a given insecticide must govern to a large extent the efforts which will be necessary to reduce the residue of that particular insecticide to a safe level. Acute, chronic, and dermal toxicity must all be considered by the toxicologist. Of these the chronic toxicity is probably the most important from the standpoint of the food processor. Information on chronic toxicity takes much longer to obtain than information on acute and dermal toxicity and is more difficult to interpret. Continuous observations extended over a period of several years are needed in order to obtain information on the chronic toxicity of a given insecticide in respect to certain experimental animals. Having this information, it is then necessary to interpret the findings in terms of the possible toxic effects on human beings. This is a problem of the utmost importance and one giving rise to a great deal of uncertainty. In weighing the evidence it is necessary to consider clinical observations on human beings who have been exposed to the insecticide over various periods of time. The variability of human response to a given insecticide must also be considered, since those individuals who happen to be particularly sensitive to a particular toxic substance must be afforded protection as well as those of average sensitivity.

As a result of extensive pharmacological investigation Dr. A. J. Lehman, Chief of the Division of Pharmacology of the U. S. Food and Drug Administration, has presented data on acute oral toxicity, toxicity by dermal application, and chronic toxicity of many of the new organic insecticides on rats and on dogs *(8)*. Dr. Lehman at a meeting of the American Chemical Society in San Francisco on March 29, 1949 amplified his previous statements to include maximum safe residual limits for the new organic insecticides *(9)*. These limits are based upon present toxicological data and are subject to modification as more information becomes available.

The publication of these residual limits does not imply the existence of official tolerances of the U. S. Food and Drug Administration for the presence of insecticide residues in processed foods, since the present Food and Drug law prohibits the presence of any poisonous substance in any quantity whatever, regardless of potential injury to health, unless the addition of the substance is required in the production of the food or cannot be avoided by good manufacturing practice. It must be established, therefore, that the use of an insecticide is required and can not be avoided in the production of a food and that good manufacturing practice is unable to remove all traces of it before an official tolerance can be established. The law further states that in such cases legal tolerances can be established only after public hearings have been held.

The manufacturer of insecticides is continually searching for new compounds which give more effective control of insects. These investigations must take into consideration the possible toxicity hazards to human beings and domestic animals connected with the use of new materials, and manufacturers should adopt careful screening technics in order to eliminate those materials for which adequate safeguards can not be developed. Manufacturers should also realize the importance of performing studies on chronic toxicity before new materials are placed on the market. Unfortunately, as previously indicated, these studies take time and immediate advantage can not be taken of a new discovery in the field of insecticides, however meritorious the new material may ultimately turn out to be.

The Food Technologist and the Spray Residue Problem

It must be the concern of the food technologist to establish to his own satisfaction that insecticide residues are being satisfactorily removed in the preparation of his product. If the insecticide is essential to the production of the raw material and if it can not be completely removed in the manufacturing procedure, he must be

sure that the residue is reduced to the lowest possible limits consistent with practical plant operation.

The knowledge of the extent to which a raw product is contaminated with a toxic material must serve as a guide to the food technologist in deciding what procedures can be used in preparing his product. For example, if a processor is receiving apples, which are contaminated with lead arsenate, for manufacture into applesauce, the particular manufacturing procedure he is using governs to a large extent the amount of residue that can be tolerated on the raw fruit. If the apples are to be completely peeled and cored, considerably larger amounts of residue can be tolerated than if the apples are to receive merely a water wash prior to pulping.

An acid and/or detergent wash may serve to remove a considerable portion of the residue, but even this may not be sufficient to reduce the level of residue in the final product to satisfactory levels if the initial contamination is too high.

Looking at it from another standpoint, the presence of objectionable amounts of residue on a raw product may require the manufacturer to use methods of residue removal which are not economical and which may result in a somewhat less desirable product. Referring to the example quoted above, complete removal of the skins and cores of apples contaminated with lead arsenate increases considerably the costs of manufacture of applesauce and such a procedure is thought by many to result in a less desirable product, since the skins of apples contribute certain characteristics of color and flavor which are said to improve the quality of the final product.

Recent investigation has shown that removal of the skins of various fruits and vegetables either by peeling or by screening removes essentially all of certain insecticides such as DDT and DDD (TDE) since the residues remain in the peel or pomace *(11)*. It is apparent, therefore, that, up to a certain point, residues of certain of the newer insecticides can be tolerated on raw products such as apples, pears and tomatoes, if the peels are to be removed either by peeling or screening. Other insecticide residues such as arsenic, however, are not as highly concentrated in the pomace *(10)* and must be reduced to a much lower level prior to pulping and screening in order to avoid contamination of the finished product. It must be borne in mind also that other products such as apricots are usually processed without removal of the skins, and that the fragile nature of certain of these products renders vigorous washing undesirable.

The presence of an insecticide residue may affect the quality of a product through the production of off flavors and odors, and possibly through diminution of the natural odor and flavor of a product. It is now well known that certain technical preparations of benzene hexachloride cause the development of off odors and flavors in a number of products *(7)*. The effect that other insecticides might have on the quality of a product is not known with too high a degree of certainty since changes produced by an insecticide may be of such a subtle nature that they may very easily be overlooked. Those changes may well be within the range of normal variation of a given product and be undetectable except by comparison with an uncontaminated control sample, yet their over-all effect may be a general lowering of the quality of a product. The food technologist must always take into consideration the possibility that a new insecticide may affect the quality of a product and be ever on guard against the use of new and unproven materials until adequate tests have been performed.

In situations such as are noted above it is seen that the presence of insecticide residues on a product may significantly affect the economy of operations and the quality of the product produced. The benefit to both the grower and processor of insecticides in the form of increased yields and insect-free product can hardly be disputed; however, the advantages to the grower of the unrestricted use of an insecticide must be weighed against the added cost and the serious difficulties to the processor which may be brought about by the presence of objectionable residues on the raw product. The obligation of the entomologist, therefore, to develop insect control programs involving the presence of minimum amounts of residue at the time of harvest takes on added significance, and it is equally important that this information be put to practical application by growers of the raw product. The manufacturers of insecticides can perform a highly worthwhile service in the search for new insecticides which, although providing adequate insect control, are less toxic to human beings and animals and have no deleterious effect on the quality of foodstuffs.

The food technologist must, in turn, be aware of new developments in the field of insecticides, and be able to correlate this information with his knowledge of the requirements of processing and quality control.

The Analytical Chemist and the Spray Residue Problem

In order that the extent of contamination of a product with a toxic substance may be evaluated, adequate analytical methods must be developed. These methods must have the necessary sensitivity and specificity to detect extremely small amounts of the toxic substance in the presence of large amounts of extraneous material. Progress in this field must necessarily be slow and a great deal of work must be performed by the analytical chemist before a reliable method can be developed. Furthermore, a method must be checked by a number of laboratories and under a number of diversified conditions before it can be regarded as completely reliable under a variety of circumstances.

Progress to date on analytical methods for the newer insecticides has been somewhat disappointing, although not through lack of time and effort spent. The major emphasis has been placed on methods for the determination of DDT, and as a result a few fairly satisfactory methods have been developed. In our experience the colorimetric method, involving nitration and the development of a magenta color in the presence of methanolic potassium hydroxide, has been the most successful in determining small quantities of DDT in a number of products provided that suitable purification procedures are used on the initial extract. In this con-

nection the acid extraction technic of Schechter, Pogorelskin, and Haller *(12)* has been very satisfactory, although adding considerably to the labor involved in the determination. We have also found the method for parathion of Averell and Norris *(1)* to be quite satisfactory, and somewhat less laborious than the colorimetric method for DDT. Although these methods appear to be fairly reliable, it would be very desirable to have methods that are simpler and less laborious.

Methods are being developed for benzene hexachloride, chlordane, and methoxychlor *(4)* and a method has been proposed for the compound "118" *(3)*. However, these methods have not been used extensively enough to justify comment at this time, and specific methods for other insecticides such as the chlorinated camphenes have not as yet been developed.

Mention should be made at this time of the various bioassay methods for detection of insecticide residues. Such methods have been developed using house flies *(6)* or mosquito larvae *(5)* for the detection of toxic substances and provide highly sensitive and possibly even rapid means of detecting these residues. These methods are of great value in detecting residues for which no sensitive chemical method is available, and for providing confirmation of the results obtained by chemical methods; however, they can not be expected to be specific for a given insecticide or to provide highly accurate results.

Since many of the newer materials contain chlorine, a measure of the amounts present may be obtained by total or labile chlorine determinations provided that the residues can be separated from other chlorine-containing compounds. These procedures are of great value in providing rapid control methods for checking the possible presence of insecticide residues and are the only known chemical methods for determining many of the newer insecticides, but they can not be regarded as the final answer to the problem since they do not, except in special instances, distinguish between the various organic chlorine-containing compounds which may be present in a given sample. Further efforts must be directed toward the development of specific methods for individual insecticides and for better and more rapid means of separating individual insecticides from interfering substances.

Much of the responsibility for the development of analytical methods for new insecticides probably should rest on the insecticide manufacturer, since an investigation of the toxicity hazard connected with the use of a new material can not be completed until an adequate analytical method for that material becomes available. The cooperative effort of many individuals is required, however, in the checking and ultimate refinement of an analytical method, and this type of work should be given high priority by all those organizations seeking to investigate the spray residue problem.

Suggestions for Conducting Residue Studies

Of primary importance to the food technologist is the problem of determining under a given set of circumstances the amount of residue that will remain on the final product. Much work already has been done on this problem and there have been many hundreds of analyses performed on a great many products. Much of these data, however, are difficult to interpret both through improperly controlled experiments and as a result of inadequacies in the analytical methods which were used.

There is a great need for further data on insecticide residues and on the efficiency of methods of removal of these residues by various commercial procedures. Some suggestions relative to means of obtaining this information and a few words of caution in the interpretation of results may be pertinent to this discussion.

1. Studies should be performed on raw products of known field history. Residue studies should be confined to those insecticides of proven effectiveness in the control of the particular insects encountered on a particular crop, and the best entomological advice should be obtained as to times and rates of application. In this connection it is suggested that, in order to obtain the best advice and the widest circulation of results, these experiments be performed under the control and supervision of the various state universities and experiment stations rather than by private agencies. Although it may be desirable to exaggerate conditions somewhat in order to obtain the worst conditions in respect to residue that may be encountered commercially, it is doubtful whether dosages in excess of those recommended by competent entomologists should be used.

2. The residue content should be determined at the time of harvest before any steps have been taken to remove that residue. This value can be taken as a measure of the success of a particular field treatment in respect to attaining the minimum amount of residue.

3. The efficiency of residue removal brought about by a certain procedure or by the use of a particular piece of equipment should be determined by analyses performed on the raw or partially processed product immediately before and after the particular treatment. In evaluating the efficiency of a commercial cleaning procedure, however, the following factors must be kept in mind:

(a) The equipment must be operated at full capacity and enough raw product must be run through it to establish equilibrium conditions. This usually means that a ton or more of raw product is required.

(b) The initial contamination of the raw product must be taken into consideration in evaluating the efficiency of a given operation since experience has shown that a higher proportion of residue is removed from a highly contaminated product than is removed by the same operation performed on a product of lower initial contamination.

(c) The previous history of the raw product must be considered. For example, it has been found that cold storage pears form, during storage, a wax coating over pores in the surface of the pear, thereby trapping whatever residue may be present in those pores. It may be expected, therefore, that the efficiency of a given wash would be higher for "hot" pears than for cold

storage pears having the same level of contamination. It may also be mentioned that insecticide formulations may contain binders or stickers which make the insecticide stick more firmly to the raw product and consequently be more difficult to remove.

(d) Some materials penetrate into the tissues of the plant and can not be removed by any treatment that can be given to them. Stripping of the residue by solvents such as is customarily used in determination of the surface residue on raw fruits and vegetables may in certain instances give an erroneous impression of the amount of residue actually present. For example, olives have been found to have absorbed 11 to 12 p.p.m. of DDT eight months after treatment *(2)*. Benzene hexachloride is known to penetrate into the tissues of certain products with the production of an off flavor.

4. The effect of heat processing or storage on insecticide residues must be considered. Certain of the organic materials undergo partial destruction during heat processing *(13)*; however, little is known regarding the decomposition products of these materials. For the most part these decomposition products have not been identified, their toxicities have not been determined, and methods are not available for their estimation.

5. Only the most reliable analytical methods should be used in residue studies and the analytical work should at all times be under the supervision of competent analysts. It has been suggested that authentic test material should be checked by more than one laboratory, in order to assure the reliability of the analytical results. It may be desirable in some instances to postpone a complete investigation of a new material until a suitable analytical method is developed, since results obtained by inadequate methods may be entirely worthless and even misleading.

In evaluating analytical results the accuracy and the sensitivity of the method must always be considered. When "trace" or "zero" values are reported they must always be considered in the light of the sensitivity of the method, and this degree of sensitivity probably should be reported when "trace" values are recorded. It must be borne in mind that a "zero" value does not necesarily mean that a toxic material is absent from a product but merely that there is too small an amount present to be detectable by the particular method employed. It is quite possible that once certain insecticides have been applied to a product they can never be completely removed even though the amounts present may be considerably less than the smallest amounts that can be detected by any known method.

Residue Studies on Raw and Processed Products

During the past two seasons our laboratory has conducted studies on the residues of DDT, DDD, and other materials on tomatoes in cooperation with Dr. Michelbacher and his associates at the University of California *(10)*. The results show that residues of DDT and DDD on the raw tomatoes obtained for these studies immediately after dusting and before washing ran in the neighborhood of 0.5 to 1.0 p.p.m. and never higher than 2 p.p.m. Ordinary washing served to remove a considerable portion of this residue and removal of the skins and cores either by peeling or by screening served to remove a large proportion of the remaining residue, with the result that the amounts of residue which remained in the finished product were usually very close to the limits of sensitivity of the analytical method. The data also indicated that certain other new insecticides recommended for use on tomatoes will behave similarly to DDT. The indications are, therefore, that there is little to be concerned about, for the present, as far as insecticide residues on tomatoes are concerned.

The situation is quite different, however, when the same insecticides are applied to apricots. This fruit is usually consumed without peeling, has a fuzzy surface which tends to hold the residue, and furthermore its fragile nature will not permit too severe a washing treatment. Our laboratory conducted experiments during 1948 in cooperation with A. D. Borden of the University of California on apricots dusted with DDT, DDD, parathion, and lead arsenate. The results show that DDT, DDD, and lead arsenate will resist weathering for a considerable length of time. Our results also indicate that ordinary washing procedures are entirely ineffective in removing these residues, which remained in easily detectable amounts in canned unpeeled apricots. Parathion could not be detected after a two-month weathering period. It remains to be established, however, how much time under any set of conditions is required for the disappearance of detectable amounts of this material and whether there is any danger of absorption of the material into the fruit. The results indicate that more work is needed on this and similar products.

Summary

Further progress on the solution of spray residue problems confronting the food processor will depend largely on the degree of cooperation which can be established between such organizations as the insecticide manufacturers, the university and experiment stations, and the various industrial laboratories concerned with food production and processing, and also upon the ability of these groups to distribute the information which they are able to gain where it will do the most good.

The specialized knowledge of the entomologist, the toxicologist, the analytical chemist and the food technologist, as well as other scientists, must be applied to the solution of the various problems connected with spray residues, and the information gained by these groups must be given wide circulation among farmers, crop dusters, food processors, and insecticide dealers and manufacturers. An understanding of the various federal and state laws pertaining to economic poisons and toxic residues is essential to all food processors and producers.

Further research is needed on methods of insect control involving a minimum of residue at the time of harvest, on chronic toxicities of the newer insecticides, on simpler and more specific analytical methods for toxic materials, on the effects of insecticides on quality,

and on the efficiency of various methods of reducing the residue content of processed foods.

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Some Applications of Food Technology to the Definitions of Label Terms for Canned Foods[a]

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(REVIEW PAPER)

The practical applications of science extend themselves in many directions and sometimes to seemingly remote ends. One of these seemingly remote ends is the foundation provided by food technology for informative labeling—specifically a standardized glossary of label statements for the "quality" factors of canned fruits and vegetables. To this specific end and its essential partner, quality control, some of the findings of scientific research have been adapted and applied to the measurement of factors of eating quality. Without technical methods of measuring quality factors, the marked progress in informative labeling of canned foods in the form of standardized specifications for consumer guidance would be impossible.

For a number of years the canning industry, through its national association and in cooperation with the major food distributor associations, has been developing a glossary of objectively defined label terms for canned fruits and vegetables. The motive is to provide consumers with more information than is required by law and regulation. Food technology plays a vital role in this program.

The basic problem in labeling canned fruits and vegetables is the difficult one of informing the consumer of their eating qualities which in turn depend principally on sensory reactions; the sense of taste to flavor; the sense of feel to texture; the sense of sight to appearance.

People's impressions of "bland," "mild," "sharp," "strong" flavor or of "soft," "medium," "firm," "smooth," "coarse" texture are widely variable. Direct labeling for sensory reactions would be subject to variable interpretation and hence impracticable, even if any such label terms as these could be standardized by objective definition. Flavor, for example, cannot be reliably measured or objectively defined by any known method. However, most fruits and vegetables do have traits or characteristics whose condition is closely correlated with differences in flavor, texture, and appearance and, for which traits it is possible to make standardized label declarations. When consumers are informed of the nature of a significant trait, as for instance the ripeness of fruits, then each consumer can relate the significance of that trait to his own sensory reactions. Bluntly, when the homemaker knows what is in the container she may buy what her own family likes best regardless of other people's tastes.

The most important differences in eating quality of many fruits and vegetables are caused by changes which take place in their natural growth before harvest. Nature's changes are progressively gradual rather than in abrupt jumps from one clearly apparent maturity classification to another, each readily distinguishable either for consumer selection purposes or for labeling. Nevertheless, the wholesale buyer and canner do buy and sell many canned fruits and vegetables on the basis

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of three long recognized classifications of ripeness or maturity respectively spoken of in the trade as "Fancy," "Extra Standard" or "Choice," and "Standard."

The labeling program which we are discussing translates and interprets this trade language or idiom into commonly-understood, specific, descriptive words or terse phrases for the consumer's information. For example, the very young, tender, delicately flavored stage of peas and whole kernel corn which the trade calls "Fancy" is labeled *very young*. The intermediate stage, still tender, though firmer and with more developed flavor which the trade calls "Extra Standard" is labeled *young*. The third stage, still succulent but with fully developed flavor and usually somewhat firm, which the trade calls "Standard," is labeled *nearly mature*.

The canner or distributor usually can apply each of these three label terms correctly to the matching maturity classification of his own lots, because of reasonably uniform trade concepts and judgments sharpened and tempered by experience. Most sales at wholesale are consummated on taste, smell, feel and sight of samples. Even among the experts differences of opinion do sometimes occur though often they are on shadings with which the average consumer would not be too concerned even if recognized.

For the purpose of labeling, however, it is imperative that these label terms do not overlap their proper limits to the extent that the overlap in sensory reactions, that is in eating quality, would be significant to the consumer and hence misleading or confusing. For this reason, limits for each label term must be established and then standardized by some objective determination. It is here that food technology steps in to play its vital role.

The established procedures for developing the label terms for any product can best be told by continuing the references to peas and whole kernel corn. Many canned samples of these vegetables were examined by committees of experts for several seasons. Differences of expert opinion as to proper placement in the three maturity classifications were reconciled by mathematical scoring and by special consideration of borderline and disputed samples. Duplicates of these samples were measured in the laboratory for their starch content by the Alcohol-Insoluble Solids test which was originated through the research of Dr. Z. I. Kertesz. Happily there is a close correlation between the composite sensory judgment of the experts and the progressive increase in the amount of starch as these vegetables grow and advance in development. The A. I. S. extraction determines principally the amount of starch and the A. I. S. test was appropriated as an objective measurement for the stages of maturity for these vegetables. The percentage of alcohol insoluble solids which match the borderlines of the organoleptically determined classifications were set as the definitive standards for the label terms *very young*, *young*, and *nearly mature*.

The author *(6)* has more completely discussed the fundamentals of labeling procedures elsewhere, but this discussion of peas and whole kernel corn illustrated the principles of this program, the method of developing it, and the dependence for reliability in this and in every labeling program upon objective measurement methods for characteristics correlating with eating qualities. In more cases than not, the measurement standards for label terms have been first published as tentative, awaiting the accumulation of experience before finalizing. Some of the problems involved in the matching of objective measurement data with subjective judgment have been capably discussed by Plank *(7)*.

The dependence on objective measurements has a corollary in that reliable labeling in this program or any other is limited to characteristics or traits for which objective measurement methods can be found. In some cases nature has been stubborn in yielding to objective tests and this program at present does have limitations. Though the A. I. S. test is applicable to whole kernel corn, it has not been found reliable for cream style corn and no other reliable test having been found, there are no labeling recommendations for the maturity of cream style corn. It has been agreed that these three label terms are appropriate for green and wax beans but again no method of measuring their maturity has been uncovered.

Characteristics of importance are not the same for all products. In tomatoes, for example, the consumer is concerned not only with ripeness but with the solidity of the tomato meat. After extensive consideration it was concluded that the most practical way to declare the condition of the tomato meat was in terms of wholeness by three label statements: *whole*, *mostly whole* and *not whole*. It is admitted that objective methods of defining wholeness need improvement but the factor of wholeness is important in this major canned item. Faced with this realism of practical importance, an experimental exception to the principle of objective measurement was made in the form of specific and precisely worded definitions of these three label terms. If experience proves this to have been a mistake, it will have to be corrected.

The simplest, most practical, most informative method of labeling for ripeness of tomatoes is in terms of red color. Three label terms are recommended: *uniformly red*, *mostly red*, and *may vary in red*, which correspond with trade classifications. Color is measured with Munsell Color Cards according to a method modified from the specifications in the Food and Drug Administration Standard of Quality for canned tomatoes. A second and correlated method was independently developed which is the spectrophotometric measurement of transmitted light at stated wave lengths through a clear extract of the pigment from the samples. Suitable ratios and maximum variations for both methods were established between portions of the samples whose color might be variable to the naked eye.

For the labeling of beets, spectrophotometric measurement of transmitted light through the normal liquid of canned beets into which the beet color bleeds is used as the standard for three label terms: *deep red*, *bright red*, and *light red*.

Several years ago it was suggested that the ripeness of peaches and apricots might be measured by the spectrophotometric determination of the amount of green pigment present because, as everyone knows, the visible green disappears progressively as these fruits ripen.

For three years experimental canned samples were prepared from the important commercial varieties of these fruits, from the commercially important growing areas, and at successive stages of development. Following established procedures these samples were judged and rated for ripeness by several committees of experts. Duplicates of the samples were measured in the laboratory by an empirical method developed for the purpose. The preliminary work and the method have been discussed in detail by Kramer and Smith *(4, 5)*. Briefly the method consists of preparing a dilution of the ether extracted pigments from a standard portion of the comminuted drained fruit. The green pigment in this solution is measured by the Beckman Spectrophotometer at a particular wave length. During these years of experiment, the yellow pigment was also measured but these measurements did not prove reliable. The green color measurements proved closely related to the gradations of ripeness as judged by the committee of experts.

The reliability of the method was further tested against the known phonomenon that fruits progressively ripen on the tree. The measured green color of peaches picked from the same trees at successive dates was found to diminish progressively. The stability of the color was tested by holding canned samples in storage at different temperatures for a year. Color measurements recorded no significant changes in green pigment. Another year was devoted to the measurement of regular cannery line production samples.

After four years' study which indicated that the method of measurement is reliable, spectrophotometric limits were tentatively set this last spring for the label term *ripe*. Spectrophotometric limits were also set for the label term *may vary in ripeness* which well describes the less carefully selected but acceptable economy packs which the trade calls "Standard."

Because the limits for each label term are based on well established trade understandings, canners and distributors seldom feel the need of scientific measurements to apply these label terms to their own packs. Measurements may become important in borderline cases and it is interesting to observe that the most experienced are the most anxious to have their judgment checked.

Spectrophotometers are expensive instruments. Few canners are equipped with them or employ trained technicians who can utilize them. Preliminary and very limited investigation indicates the possibility of developing a comparatively inexpensive photometer equipped with a chromatic filter for specified wave lengths which could be manipulated by a normally intelligent person with but limited training and would give readings equivalent to the spectrophotometric readings. However, until there is such equipment and when necessity arises, the average canner or distributor will have to submit samples to a properly equipped laboratory.

This illustration points up the need for methods and techniques which are both simple and reliable. Progress has been made in that direction. The specific gravity of peas increases with natural growth. Quite accurate determination of the maturity of canned peas can be made by flotation tests in different concentrations of salt solution. When work is completed in correlating brine flotation tests with the basic A. I. S. test, standards for the three maturity label terms will no doubt be supplemented by simpler brine flotation standards.

Only recently a mechanical device was developed by Wilder of the N. C. A. Western Branch Laboratory for the determination of the presence or the absence of tough fibers in canned asparagus—an important factor. It will no doubt prove valuable for the purpose of objectively measuring whatever the basic level may be in the anticipated Federal Standard of Quality for asparagus and perhaps be useful for additional voluntary labeling.

Quality controls during production are justified in their own right, but the necessity and value of production quality control is increased if the specifications of the important factors of the product are to be given on the label. Maturity and the closely correlated eating quality of the succulent vegetables cannot be manipulated after harvest. Intensive field observation, temperature records, and control of the time of harvest can and do control the stage of succulence and consequently the eating quality to a great degree. Beyond that, however, uniformity as regards maturity is achieved in the cannery by sorting the raw fruits into maturity classifications. Maturity identification, however, is a prerequisite to sorting.

The resistance of raw peas to shearing provides a reliable identification of maturity when measured by the tenderometer, a shearing device invented and patented by Dr. W. McKinley Martin, formerly of the American Can Co., and made available by his assignee to the industry without royalty. The tenderometer is easy to operate. Standardized measurements correlate closely with A. I. S. tests of the canned product, hence it is valuable in identifying and combining like maturities, both of which steps are preliminaries to labeling for maturity.

Refractive index measurements which indicate sugar content are reliable determinants of maturity of raw sweet corn but again, the equipment is expensive and the method complex. Based on the fact that the liquid content of raw corn progressively decreases as corn develops, a "succulometer" has been devised which mechanically squeezes out and permits measurement of the liquid from a measured quantity of raw or canned whole kernel corn. This device is still in the experimental stage in the hands of a number of canners.

Lighting studies have been made to determine the optimum kind and quantity of light under which to sort fruits for ripeness and to detect blemishes before canning.

In the case of some items, such as apricots, peaches, pears, green and wax beans, among others, the matter of appearance depends to a considerable extent on the presence or absence of nature's blemishes and defects of different kinds, degrees and sizes. A great deal of time, care, and patience was put into a series of color transparencies of apricots—defects being photographed in the raw fruits and in the identical fruits after processing. Careful specifications were drawn up of tolerances based on kind, extent, and number of photographed

blemishes and defects. Several canneries have been experimentally using sets of these color transparencies in search for proper standards for label statements to declare workmanship. Illustrations for the same purpose are now being prepared for canned pears. This technique is a great advance in the positive definition of the kind and character of blemishes and defects. Tolerances for permissible inconsequential or minor defects and the exclusion of major defects can be readily set when and if the defects themselves can be accurately identified and classified.

The foregoing has been an outline of procedural steps which are taken for each product separately to develop this program of specification or standardized descriptive labeling. There has been enumerated a limited number of examples of the borrowings from research or from mechanical principles to effect standardization in the form of objective measurement methods. These latter have been more fully enumerated and described by Smith *(8)* and by Kramer *(3)*.

The labeling for any one item originates with a representative committee of canners of that item under the supervision of the Labeling Committee of the N. C. A. A Technical Advisory Committee, consisting of scientific and technical men in the industry, advises on scientific and technical matters. In selecting actual label phraseology, some extensive consumer research has been conducted to find words most widely understood and self explanatory. Before final recommendations are made all labeling proposals are laid before a committee representing the major distributor associations whose suggestions and criticisms are heeded.

When finally formalized, the label terms and their objective definitions are published in a labeling manual which is widely distributed to the entire trade *(1, 2)*. These recommendations carry the joint approval of the national associations, representing the canners, wholesalers, retailers, chains, and food brokers.

The development of further recommendations depends on new scientific research, and in the field of food technology—on the ingenuity to make additional adaptation from the present findings of science, to adapt new findings to practical use and on ingenuity in devising mechanical methods. It is the declared intent of the National Canners Association and its Labeling Committee to extend this program to include every fact which "is of genuine interest to consumers" and which can be "objectively measured."

This labeling program is voluntary. The label terms are offered to canners and distributors as formal recommendations of the sponsoring associations. It must stand or fall on its own merits. It is gratifying that a significant number of canners and distributors, large and small, now employ on their labels the recommendations so far made and that a large number employ most of them without the mandatory dictate of law or government regulation. That this labeling is being employed by an increasing number of canners and distributors is because the program is being increasingly recognized in principle and in practice as usefully informative and reliable for the consumer; sound and practical for the trade; and misapplications of any part of it readily enforceable under existing food laws.

Without the technological adaptations of scientific research and the employment of mechanical principles, no reliable informative labeling program such as this could be constructed.

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Hygroscopicity of Sugar and Other Factors Affecting Retention of Quality[a]

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The protection of staple food products during storage is typified by the care given sugar. Studies which have given the basis of present day care are reviewed with particular regard to changes induced by atmospheric moisture. Granulated sugars exposed to a humidity below 60% contain less than 0.03% moisture and this is favored to avoid a lumpiness referred to as caking, whereas unlimited exposure to humidities over 85% will liquefy pure sugar. The use of moisture vapor barriers in the package construction, and of caking inhibitors added to the sugar, are discussed; these measures minimize caking in the instance of fine grain sugars to a point equalling results with unprotected coarse grain sugars. Reasons for differences among the various types of sugars are attributed largely to crystal or grain size differences. The retention of other desirable characteristics of granulated sugars, and special care of Brown type sugars are mentioned; Brown sugar requires a surrounding conducive to retaining its natural moist condition.

The importance of protecting our food products during storage and shipment is a subject which has received

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unusual attention in the past ten years. This attention has resulted not only in greater emphasis on existing packaging practices, but has forced development of new techniques and new materials. To those of us involved primarily with packaging and handling food products, this recent public interest in the subject is perhaps a very small good that came from the recent war.

In the sugar industry, we have seen a wide acceptance of packaging materials and storage procedures that assist in maintaining quality characteristics. Though sugar is traditionally a stable product, not subject to spoilage, present-day industrial and domestic uses of sugar make quality retention one of our principal problems.

The value of sugar in terms of price per pound, however, is a low figure which does not permit use of currently popular but somewhat expensive packaging media, and reliance, therefore, is usually on desirable storage procedures. These procedures have been described in bulletins available to our sugar trade for some ten years or more, but the studies and reasoning on which the procedures are based is information which has received little publicity. Success that has been attained when the procedures have been used prompts the conclusion that this background information could prove useful in dealing with similar food products.

The chief problem in keeping sugar in storage is to retain the free-flowing condition of this granular product. A lumpy condition, which the industry terms "caking," has several objections. These objections range, on one hand, from the extra time taken when a customer dumps the contents of a bag into a screen-covered hopper, to the trouble caused when a customer tries to blend a dry-mix with other ingredients.

This discussion will deal with the data which prompted establishment of a 65% relative humidity as an upper safe limit for white sugars, and will comment on the effect which particle or crystal size of the product exercises on the severity of caking. Mention will also be made of other manners in which quality is impaired: the development of color and of inversion. Problems with various white sugars, ranging in type from a pulverized product to one of coarse, individual crystals, will be contrasted with problems in handling what the trade knows as Brown or "Soft" sugar.

Use of moisture-vapor barriers, and caking inhibitors added to the sugar, will also be described, with regard to why they are effective and what limitations surround their use.

Moisture Content: Principal Factor in Retaining Quality of Sugar

Although this discussion does not involve the processes in nature which combine carbon dioxide and water to form sugar, it is pertinent to mention that in extracting our sugar of commerce from the sugar cane or beet in which it is found, there is a time limitation within which we must work. The sucrose which can be crystallized into our sugar of commerce ceases to exist as a crystallizable product unless it is isolated from the other plant constituents, including the water in the cellular structure of the plant.

Once we have isolated the sucrose in crystalline form, we can avoid this deterioration or inversion, and retain its other characteristics most easily, if we can confine its moisture content to low values. Knowledge of the behavior of sugar exposed to different atmospheric humidities is therefore essential.

Early references to the behavior of sugar exposed to atmospheric conditions were somewhat limited. Browne *(1)* in 1912 related his observations on samples exposed to room conditions. In 1929 the author conducted a series of tests in which samples of granulated sugar were exposed to the range of humidities ordinarily experienced. The data obtained have proven very useful in the analysis of storage problems, and are discussed hereafter in a subsequent section.

The literature has in recent years provided certain specific data, notably the critical humidity value at which it is believed pure sucrose crystals change from a theoretically moisture free condition to a dissolved or liquid condition. Whittier and Gould *(12)* observed the humidity over a saturated solution to be 77.4%, and Dittmar *(2)* presents graphical data placing the humidity value at about 83%. An examination of the data by Keller*(4)* suggests something more like the 77.4% value. The author's work in 1929 as well as that done since with saturated sugar solutions, emphasizes that the purity of the samples at hand and the existing temperatures play a part in setting the precise humidiy value. Moreover it appears more likely that values over an 80% relative humidity apply, and that 85% is a value which under ordinary room temperatures very closely satisfies most conditions.

These experimental values presume, of course, that temperatures of the sugar and air are identical. If, on the other hand, the sugar was a few degrees colder, however, it will be affected by a humidity which is greater than the atmospheric humidity. The "effective humidity" as it can be conveniently termed, deviates very approximately two units or percent for each degree Fahrenheit difference in temperature. Corrections, either for a warmer or colder relationship can be determined with sufficient accuracy for most work on a suitable nomograph such as that issued in Data Sheet form by TAPPI *(9)*. Calculation by other methods is also possible.

The moisture content of commercially refined sugar is a barely perceptible value when in equilibrium with humidities below the critical range. The presence of moisture under these conditions is in fact believed due to the traces of what are termed non-sucrose, or melassigenic substances including invert sugar. The substances are present in such small amounts, however, that the moisture associated with them is correspondingly low, commonly between 0.01% and 0.02%. This circumstance for sugar contrasts sharply with other carbohydrate foods such as wheat flour and starch, for example, with moisture contents of about 5.00% at low humidities and over 10% moisture at high humidities. Gallay and Bell *(3)* and Wilson and Fuwa *(11)* have presented data on these products.

These low moisture values for sugar, however, show a sharp break when the exposure involves atmospheric

humidities over 75%. Observations by the author were made with small samples (approximtaely 10 grams) placed in aluminum dishes and freely exposed to known humidities (between 30% and 80%) in large glass jars stored at room temperature. Laboratory-size desiccator jars were used, the dehydrating agent being replaced by sulfuric acid solutions of predetermined concentrations according to Wilson *(10)*. The hoped-for relative humidity in each jar in the series was checked periodically by a dew-point method; a silvered test tube permanently inserted in the cork stopper of the glass cover provided the medium on which to observe the formation of a faint deposition of dew. About 5 cc. of ether placed in the test tube provided the dual means of (a) lowering the temperature of the silvered tube (through evaporation induced by an aspirator) and (b) of immersing the thermometer used in determining the temperature of the test tube surface. Readings were taken at the moment the dew first appeared and again at the moment the dew disappeared. The dew point was presumed to be the average of the two temperatures thus noted.

Moisture content values obtained in this work are shown graphically in the accompanying chart. It will be noted that the values sugest a sigmoid-shaped curve, with practically no moisture at low humidities, and that there is a rapid change in the range between 60% and 80% humidity. The trace of non-sucrose impurities present in the commercial refined sugar is a component that tends to absorb moisture gradually, and the sucrose theoretically would show an abrupt change. Thus the moisture content of the composite commercial product is in a sense a compromise or average of the two constituents. Though outside the scope of this discussion, it is pertinent to note that differences exist in the nature of these non-sucrose impurities, and in the trace amount present in the commercial sugar. Both variables will influence the humidity equilibria values over the whole range, and in particular will affect the slope of the curve or abruptness of the moisture increase in the critical storage range between 60% and 80% humidity.

Severity of Caking: Result of Dampness and Other Variables

Granulated sugar is produced as a free-flowing product, comprising a mass of individual, substantially perfect crystals. If kept dry, that is, with a moisture content below 0.02%, it will remain free-flowing indefinitely. In the event an excess of moisture is absorbed, however, and the content rises from 0.02% to 0.06% for example, the product is in danger of caking. The excess moisture is confined almost, if not entirely, to the surface of the crystal structure, and the contact between crystals is made intimate by a fusing of the syrup film on the crystals. This film is usually imperceptible, though its presence will cause the sugar mass to flow sluggishly and to assume a steep angle of repose.

In this damp condition the sugar has not caked, though a slight "set" may exist which is evident to one accustomed to the circumstance. Caking occurs after, and only after a moist sugar has lost the excess moisture, and the syrup film at the fused points of contact has resolidified on the crystal surface.

The degree or severity of caking, experienced in actual packages tested both under controlled humidity and in field observations, has been found to be a direct function of several distinct factors:

1. The moisture absorbed—which is primarily the result of a high initial humidity, but may also occur if a cold sugar is placed in a heated storage area in the winter months.

2. The points of contact between crystals—which become greater and hold more securely if the crystals are very small and if the sugar mass includes a distribution of crystals of assorted sizes.

3. The pressure imposed by a pile of sugar in flexible packages—which not only increases the points of contact, but makes more certain the fusing of any syrup film on the crystals.

Though caking is an end-result of drying a moist sugar, the severity of caking has been amply demonstrated to be a function of how moist the sugar had been. Conversely, the severity of caking apparently bears little or no relation to low humidities, provided, of course, that the "drying" humidity is in fact low enough to drop the sugar to its normal moisture content of 0.01% to 0.02%.

Once formed, a caked condition can be alleviated by re-exposure of the sugar to a damp atmosphere. This treatment will be ineffectual, however, unless the dampened sugar mass is disturbed by handling the packages sufficiently to dislodge the re-softened points of contact between the crystals. Simultaneously, the rehandled sugar must be exposed to a drying atmosphere, since the points of contact will otherwise be reunited. Rehandling, incidentally, will avoid caking in the first instance if it can be done at the time a moist sugar begins to dry.

Caking: Preventive Measures Applicable to the Product

Reference has already been made to the value of disturbing a pile of sugar that has inadvertently become damp, in order to dissociate the syrup-fused points of contact between the crystals. But this is ordinarily impractical, not only because of the physical task of rehandling the packages, but because this must be done at the start of the period in which the sugar dries.

In the instance of granulated sugar, a measure of control over caking exists if one can choose a favorable product from the standpoint of crystal size. With a controlled crystallization process in the refinery, and with subsequent screening of the sugar prior to packaging, it is possible to obtain a product that meets both appearance and use requirements and which will consistently exhibit less caking tendency. The practicability of this approach towards caking prevention was first demonstrated in caking tests made with selected series of almost identical sugars; although successive samples in a series appeared identical to the eye, the severity of caking under adverse storage conditions differed, and was found to be closely correlated with analytical data

obtained on the particle size distribution in the respective samples.

Alternately, the points of contact can be lessened by mixing a finely pulverized edible starch or an inert inorganic compound with the sugar. Such mechanical separation of the sugar crystals is effective, but being insoluble, these inhibitors can not be used where the sugar product is dissolved in water at the food processing plant. Their use in a pulverized product like powdered sugar, however, is fortunately both practical and effective, because this sugar is made into various icings by adding only a small amount of water.

The choice of starch as an inhibitor, in contrast to magnesium carbonate or tricalcium phosphate, all of which can offer substantially equal relief in reducing the points of contact, can be justified on a second and distinct basis. If the packaging material used incorporates a moisture-vapor barrier the small amount of moisture that does slowly enter the dry interior of the package will be absorbed by the starch. This preferential absorption will continue until the starch has become loaded to a point corresponding to a humidity which is critical for sugar; ordinarily, however, the sugar will have been consumed prior to that time. Though as little as 3% of starch may be used, by dry weight, protection is afforded the sugar by reason of the starch's gradual absorption curve and its vastly greater capacity to hold moisture without evident change in the starch. Moss, Schilb, and Warning *(5)* have presented some interpretations and data bearing on these distinctions between the use of inert and absorbent inhibitors.

Caking: Preventive Measures Applicable to the Package

At best, shipping conditions encountered by sugar in the course of year-round deliveries offer little in the way of choice of atmospheric conditions or practical corrective measures. It is necessary therefore to protect the more sensitive grades of sugar with a moisture-vapor barrier incorporated in the bags and cartons. When this problem was attacked in 1930 we soon concluded that a multi-wall kraft paper construction, rather than the conventional textile sugar bag, offered the most convenient approach to this problem. Waxing one of the kraft sheets in the multi-wall construction was found to be fairly satisfactory but the protection was lost at the point in its use where wrinkling had cracked the wax and destroyed its protective value. In cooperation with a paper producer, a special uniformly asphalted laminated sheet was developed for this purpose. Tests with the laminated paper itself, and with completed bags demonstrated the value of uniform application of the asphalt in a continuous film. Similarly, it was necessary to use an asphalt film that, on one hand, was not easily cracked when the bags became wrinkled, and that on the other hand, was not easily forced through the pores of the paper laminae when subjected to the pressure of high storage piles. More recently Scribner, Carson and Weber *(8)*, and Nelson *(6, 7)*, have covered these points in some detail.

Placement of the asphalt ply in the multi-wall bag was governed by the desire to have a sheet or two of plain paper between the vapor barrier and the sugar. While the question of asphalt contamination entered into this selection, of equal importance was the use of the plain paper sheets to act as a "blotter" much as does starch when used as a caking inhibitor.

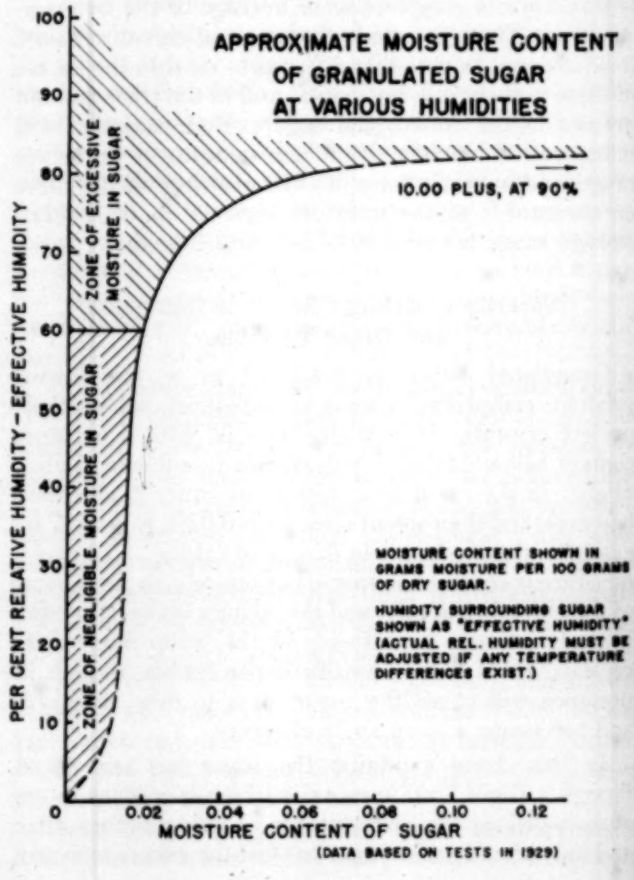
Although the application of wax to the closure seams was found to be beneficial, the cost of application could not be justified by the incremental measure of protection against water in vapor form. In the ordinary movement of sugar, we were not confronted with sea water and tropical rainfall which in more recent years prompted our government armed services to specify this added protection.

General Precautions

Prolonged dampness, as previously mentioned, will promote a deterioration or partial inversion of sucrose. Though this condition is rare in commercial experience and does not ordinarily prevent use of the sugar, it constitutes an added reason for keeping sugar reasonably dry.

Similarly, prolonged storage in hot locations, as for example, by a radiator during the winter months, can darken the color of granulated sugar to an extent which is visually perceptible to an experienced person. Temperatures below 100° F. are recommended.

A singular sugar product commonly called Brown, or Soft sugar, requires the opposite care than granulated sugar. Soft sugar has a natural moisture content of several percent, usually about 4%, and it is essential to retain this moisture in order to preserve the friable or workable texture of the product. The same package



that is used to keep granulated type sugars dry is used to keep Soft sugar moist. Supplementing this, storage humidities between 60% and 70% are recommended for Soft sugars whenever extended storage periods are apt to occur.

To summarize, white sugars such as granulated and powdered products should be stored where humidities are below 60% and the temperatures are below 100° F. Brown sugars best retain their quality under damper conditions, a humidity between 60% and 70% being preferred and a temperature below 75° F.

Temperatures appreciably lower than ordinary room conditions should also be avoided. In the instance of granulated sugar, temperature differences accentuate humidities, and in the instance of Brown sugar, freezing temperatures solidify the syrup associated with the sugar crystals and the mass becomes unworkable.

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The Occurrence of Free L-Glutamic Acid in Various Foods[a]

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Monosodium glutamate has been used for many years by the Chinese and Japanese to make their bland foods more palatable. Small amounts of this material which adds no flavor, color or aroma of its own, bring out or intensify the intrinsic flavor of meats, seafoods, vegetables or dairy products to which it is added. Although the salts of many of the amino acids have a slightly sweet or bitter taste, the monosodium salt of the dicarboxylic amino acid, glutamic acid, is the only one which exhibits this particular property of accentuating flavors.

Crocker *(1)* and Cairncross *(2)* have studied this particular quality of monosodium glutamate. They have found that monosodium glutamate is entirely without odor when pure. Its taste has all four components, sweet, salt, sour and bitter and can be matched rather well with 0.6 threshold of sweetness, 0.7 saltiness, 0.3 sourness, and 0.9 bitterness. Saltiness and sourness, which characterize glutamic acid and its sodium salt are exceptional tastes among amino acids. In addition, two other factors not ordinarily encountered, that is, tingling and persistence are also present. Whereas flavor is usually the result of two senses, taste and smell, monosodium glutamate brings into play a third sense since it has the capacity for stimulating the feeling nerves of the mouth and throat to produce the sensation described by Crocker *(1)* as a "feeling of satisfaction."

In this country, monosodium glutamate has been used for some time by chefs and gourmets interested in accentuating the flavors of their fine foods, but it has only been recently that the supply has been sufficient to make this material generally available to food processors and housewives. For a time, its use by food processors and canners was complicated by the fact that the provisions of the Federal Food, Drug and Cosmetic Act required that any material to which monosodium glutamate had been added must bear the label "artificially flavored with monosodium glutamate."

It is well known that glutamic acid is involved in the metabolism of plants and animals. If it could be shown that it occurred naturally in prepared foods, it seems reasonable to suppose that the term "artificially flavored" is inappropriate. Therefore, it was decided to determine whether or not glutamic acid existed as such in vegetables and meats which had *not* been hydrolyzed. This glutamic acid has been designated as "free" glutamic acid in order to differentiate it from any which would be bound in protein and liberated by hydrolysis. In foods, this free glutamic acid would undoubtedly be present as monosodium glutamate.

Determinations have been made of the amount of free glutamic acid occurring naturally in cooked fresh vegetables and meats and in several varieties of canned vegetables and seafoods of recognized commercial brands. Added monosodium glutamate has also been determined in an experimental commercial pack of boned chicken. Recently, the Food and Drug Administration ruled that the "artificial flavoring" designation is no longer required in canned foods to which monosodium glutamate has been added but its presence must be declared.

[a] Presented before the Ninth Annual I. F. T. Convention, San Francisco, Calif., July 14, 1949.

The superior flavor of young and of freshly harvested vegetables is well recognized. Since monosodium glutamate enhances flavor, it was of interest to us to know whether there was a larger amount of free glutamic acid present in these vegetables than in more mature ones or in those stored in the refrigerator for short periods of time before preparation.

Freshly picked sweet corn of three stages of maturity (all edible) and garden peas graded into three sizes were cooked within 6 hours of harvest and analyzed. Free glutamic acid was also determined in portions of these cooked vegetables and of raw vegetables cooked after various periods of storage in the refrigerator.

Procedure

Microbiological assay with the micro-organism *Lactobacillus Arabinosus* 17-5 has been the procedure used for determination of free glutamic acid in all the foods tested. The materials for assay were treated with 70% isopropanol according to a procedure established in our laboratory for the extraction of the non-colloidal nitrogen fraction of sugar beets.[b] Since we were interested primarily in determining the presence or absence of glutamic acid occurring naturally in prepared foods, no particular study was made to determine whether complete extraction was accomplished in the various types of foods studied.

Canned Foods: In most instances two cans of each variety of foods, purchased from a local grocery store, were analyzed. There was no indication on any of the labels that monosodium glutamate had been added. After the weights of the liquid and drained canned food had been determined, 25 g. of the drained food with its proportionate amount of liquid was mixed with 175 ml. of isopropanol and made up with distilled water to a total volume of 250 ml. This mixture was homogenized in a Waring blendor for two minutes and allowed to stand overnight and then filtered. The isopropanol from 150 ml. of the filtrate was distilled in vacuo and the residue made up to a final volume of 50 ml. for assay. Calculations were based on the 25 g. drained weight of material used.

Fresh Vegetables: 25 g. portions from a large composite of the prepared vegetables were weighed into flasks and cooked until tender in the minimum amount of distilled water. Salt was added. The mixture was cooled and then treated with isopropanol in the manner described for canned foods.

The sweet corn used for the storage experiment was a golden bantam hybrid designated as "Golden Cross" purchased from a local truck farmer a few hours after it had been picked. It was the custom of this farmer to sort the corn for sale into three grades of maturity; very young, medium, and mature. All grades were tender and had a flavor superior to most of the corn available at markets. The corn was cut from the cob of a dozen ears of each grade and thoroughly mixed. 25 g. aliquots of the raw and cooked corn were stored in closed flasks in the refrigerator. Analyses were made of the corn, raw, immediately after cooking and at 24, 48 and 72 hour intervals after storage. Duplicate determinations were made in each case and the averages of these determinations reported.

The peas were picked from an experimental plot. The duplicate samples in this case were from peas picked from different locations in the same row. They were shelled and graded roughly into very small, medium and large sizes. Aliquots were prepared and stored as in the case of the sweet corn.

Cooked Meats. *Roast Beef:* 100 g. of beef (fat removed) from the center cut of an 8½ pound standing rib roast, salted and roasted for 3½ hours at 300° F. were minced in a Waring blendor with 100 ml. of distilled water. 175 ml. of isopropanol was added to 50 g. of the mixture and the treatment was the same as described for vegetables. 50 g. of the juice which exuded when the roast was sliced was also mixed with isopropanol and analyzed. In this case, calculations were based on the weight of the juice used.

Roast Pork and Roast Lamb: Fat-free portions of the center cut of a 6 pound pork loin roast and of a 6½ pound leg of lamb roasted at 350° F. were prepared similar to roast beef.

Link and Patty Sausage: The sausages were fried over low heat until they were well done and the excess grease discarded. The skin was removed and the meat homogenized in a Waring blendor as described above.

Bologna and Frankforters: The bologna was analyzed without further cooking; the frankforters were allowed to stand for 10 minutes in water which had just come to boiling, according to the directions given on the package.

Microbiological Assay: The basal medium for assay is a modification of that of Hac, Snell and Williams *(3)*. The constituents of the medium and the method of preparation are given in Table 1. The total volume of medium and sample in each tube was 5 ml. The tubes containing the medium and sample were sterilized at 15 pounds pressure for 20 minutes, inoculated with a drop of a dilute saline suspension of L. arabinosus 17-5 and incubated at 34° C. for 64 hours. The acid produced during growth was titrated with 0.1 N sodium hydroxide electrometrically or using bromthymol blue as an indicator.

The standard glutamic acid curve was prepared using 0.01 mg. increments. The assay range is satisfactory between 0.02 and 0.3 mg. The glutamic acid values reported were the average of 6 determinations made in duplicate tubes at 3 levels of concentration. The procedure has proved satisfactory for the assay of most natural products although occasionally activators or inhibitors have caused difficulty.

Since L. arabinosus can utilize glutamine instead of glutamic acid for growth, this procedure can be adapted for the determination of glutamine also, provided sterilization of the sample is accomplished by some means other than heat. Heat converts glutamine into the ammonium salt of pyrrolidone-carboxylic acid which cannot be utilized. If the sample, sterilized by filtration through a Berkefeld filter is added aseptically to the heat sterilized basal medium, glutamine can be determined and differentiated from glutamic acid.

[b] To be published elsewhere.

TABLE 1

Basal Medium for L-Glutamic Acid Assay using L. Arabinosus 17-5

Amino Acids	Mg./L.	Purines, etc.	Mg./L.
DL Alanine	600	Adenine Sulfate	30
DL Isoleucine	400	Guanine HCl	30
DL Methionine	400	Xanthine	30
DL Norleucine	400	Uracil	30
DL Norvaline	400		
DL Phenylalanine	400	Ammonium sulfate	6 g.
DL Serine	400	Sodium acetate	66 g.
DL Threonine	400		
DL Valine	400	Salts B	15 ml.
DL Aspartic Acid	2400	$MgSO_4 \cdot 7H_2O$ 10 g.	
L Arginine HCl	300	NaCl 0.5	
L Cystine	300	$FeSO_4 \cdot 7H_2O$ .5	
Glycine	300	$MnSO_4 \cdot 4H_2O$ .5	
L Hydroxyproline	200	Water to 250 ml.	
L Histidine Mono HCl	300		
L Leucine	200		
L Lysine	300		
L Proline	300		
L Tryptophane	300		
L Tyrosine	300		

Dissolve amino acids, purines, ammonium sulfate and sodium acetate by boiling in 900 ml. of distilled water; cool and add 15 ml. of salts B; adjust pH to 6.3 with NaOH and add water to 1 liter. This is double strength medium. Store in the refrigerator.

Vitamins	Mg.	Solution A	
Biotin	0.05	KH_2PO_4	25 g.
Ca Pantothanate	2.5	K_2HPO_4	25 g.
Nicotinic Acid	2.5	Water to 250 ml.	
Riboflavin	2.5		
p-Aminobenzoic	3.8		
Thiamin HCl	6.3		
Choline Chloride	31.4		
Inositol	31.4		
Pyridoxine HCl	125.0		

Gently warm in 50 ml. H_2O. Store in refrigerator not longer than 2 weeks.

At the time of assay, for 100 tubes (final volume 5 ml.) mix together 245 ml. of amino acid solution, 5 ml. solution A, 2 ml. vitamin solution 10 g. glucose, and 30 mg. glutamine. If the samples to be assayed are less than 0.5 ml. in volume, add an equal volume of water to the above mixture; then add 5 ml. of this diluted medium to each tube containing the sample. If samples are larger than 0.5 ml., add water to 2.5 ml. and then add 2.5 ml. of the undiluted medium.

Non-Colloidal Nitrogen: In a few instances, total non-colloidal nitrogen determinations were made on the isopropanol extracts.[c] Micro Kjeldahl determinations were made using the Gunning and Arnold modification of the digestion procedure.

Dry Solids: Were determined by oven drying to constant weight.

Results

The results of the assays for free L-glutamic acid in 16 varieties of canned vegetables and 6 varieties of cooked fresh vegetables are listed in Tables 2 and 3. Any glutamine present in the vegetables would not have interfered since the samples were all heat sterilized. The highest value 0.23% free glutamic acid was obtained in canned mushrooms. All other vegetables had amounts varying from 0.17 in fresh garden peas to 0.005% in canned yellow wax beans. No glutamic acid was detectable in hominy. This might be expected since the alkali and washing treatments required in the preparation of hominy would probably remove any free glutamic acid present in the corn.

[c] We are indebted to Mr. Basil B. Dowling for the Kjeldahl and dry solids determinations, and to Dr. A. C. Walker for the isopropanol extractions.

Fresh young peas and sweet corn contain more free glutamic acid than samples of more mature vegetables from the same field (Tables 4 and 5). It may be that in vegetables, the amount of glutamic acid present is proportional to the rate of growth.

Some glutamic acid was lost during even the short cooking required for peas and corn since the raw vegetables contained a higher percentage of free glutamic acid than the cooked vegetables. However, very little loss of glutamic acid occurred in cooked vegetables stored in the refrigerator, whereas 25-35% of the glutamic acid contained in raw vegetables was lost during storage in the refrigerator. Almost all of this loss occurred during the first 24 hours.

All the canned seafoods, chicken and fresh cooked meats analyzed contained free glutamic acid in amounts ranging from 0.1% in minced clams to 0.01% in roast pork loin, pork sausage and frankforters (Tables 6 and 7). The glutamic acid content found in a commercial experimental pack of boned chicken to which monosodium glutamate had been added checked very well with the expected value.

TABLE 2

Percent Free L-Glutamic Acid in Canned Vegetables

Vegetable	Sample Identification	Total Solids	Total N	Glutamic Acid
		Percent on Drained Weight	*Percent on Drained Weight*	*Percent on Drained Weight*
Mushrooms	1	11.05	0.71	0.23
	2			.21
Green Peas	3	16.67	.75	.12
Asparagus, green	4	9.25	.59	.06
	4A			.05
Potatoes, whole, new	5			.04
Sweet Potatoes	6			.04
Sweet Corn	7			.04
Red Beets	8			.03
Okra	9			.03
Spinach	10			.02
Spinach, strained	11		.31	.02
Green Lima Beans	12			.02
Butter Beans	13			.02
Green String Beans				
French Style	14	8.67	.28	.01
Cut Style	15			.01
Strained	16			.01
Baked Pork and Beans	17			.01
Garbanza	18			.01
Yellow Wax Beans	19			.005
Hominy	20			None detectable

TABLE 3

Percent Free L-Glutamic Acid in Cooked Fresh Vegetables

Vegetable	Total Solids	Total N	Glutamic Acid	
			Sample 1	Sample 2
	Percent on Raw Weight	*Percent on Raw Weight*	*Percent on Raw Weight*	*Percent on Raw Weight*
Mushrooms, wild	11.84	1.03	0.13	0.14
Green Peas				
Small			.19	.19
Medium			.17	.16
Large			.17	.14
Pods			.04	.04
Sweet Corn				
Very young		.21	.12	.11
Medium		.33	.05	.05
Mature		.30	.06	.06
Potatoes, small new			.05	.05
Asparagus, green	6.74	.34	.04	.04
Green string beans	9.08	.28	.02	.02

TABLE 4

The Occurrence of Free L-Glutamic Acid in Sweet Corn at Various Stages of Maturity and the Effect of Storage on the Glutamic Acid Content of Raw and Cooked Corn

Treatment	Stage of Maturity					
	Young		Medium		Mature	
	N	GA	N	GA	N	GA
	Lot 1					
	Percent on Raw Weight		*Percent on Raw Weight*		*Percent on Raw Weight*	
Raw	0.25	0.13	0.37	0.08	0.42	0.06
Fresh cooked	.21	.12	.33	.05	.30	.05
Cooked after 24 hrs. refrigeration		.08		.04		.04
Cooked after 48 hrs. refrigeration		.07		.04		.03
Cooked after 72 hrs. refrigeration		.08		.04		.04
Fresh cooked		.12		.05		.05
Cooked and refrigerated 24 hrs.		.12		.06		.05
Cooked and refrigerated 48 hrs.		.12		.06		.04
Cooked and refrigerated 72 hrs.		.11		.06		.05
	Lot 2					
Raw				.09		
Cooked on the cob				.09		
Cooked after removal from cob				.08		
Glutamine:						
Raw corn	0.03%					
Cooked corn	0.02%					

TABLE 5

The Occurrence of Free L-Glutamic Acid in Fresh Garden Peas of Various Sizes and the Effect of Storage on the Glutamic Acid Content of Raw and Cooked Peas

Treatment	Size					
	Small		Medium		Large	
	(Percent Glutamic Acid on Raw Weight)					
	Sample		Sample		Sample	
	1	2	1	2	1	2
Raw	0.20	0.20	0.18	0.18	0.15	0.17
Fresh cooked	.19	.19	.16	.17	.16	.16
Cooked after 24 hrs. refrigeration			.13	.12		
Cooked after 48 hrs. refrigeration			.12	.14		
Cooked after 72 hrs. refrigeration			.12	.14		
Raw after 72 hrs. refrigeration			.14	.12		
Fresh cooked			.16	.17		
Cooked and refrigerated 24 hrs.			.16	.17		
Cooked and refrigerated 48 hrs.			.17	.17		
Cooked and refrigerated 72 hrs.			.15	.16		
Pods, raw			.04	.04		

Samples 1 and 2 were picked from different portions of the same rows.

TABLE 6

The Percent Free L-Glutamic Acid in Canned Seafood and Chicken

Food	Sample Identification	Glutamic Acid	
		Percent on drained weight	
Salmon	21	0.02	0.02
Tuna Fish	22	.02	.02
King Crab	23	.06	.06
Deviled Crab	24	.03	.02
Minced Clam	25	.09	.10
Clam Chowder	26	.03	.02
Chicken, boned No. 1	27	.04	.03
Chicken No. 2	28	.02	.02
Chicken No. 2 + 0.25% MSG	29	.23	.22

TABLE 7

Percent Free L-Glutamic Acid in Cooked Fresh Meats

Meat	Cut	How Cooked	% Glutamic Acid	
			Percent on cooked weight	
Roast beef	Standing rib	Medium rare	0.04	0.05
		Juice from slicing	.07	
Roast pork	Loin	Well done	.01	.01
Roast lamb	Leg	Well done	.02	.02
Sausage No. 1	Link	Fried, grease removed	.02	.02
Sausage No. 2	Link	Fried, grease removed	.01	.02
Sausage No. 2	Patty	Fried, grease removed	.01	.01
Frankforters		Boiled, grease removed	.01	.01
Bologna		Not cooked	.03	.03

Summary

Free L-glutamic acid has been found in unhydrolyzed samples of 15 varieties of canned vegetables, 6 varieties of cooked fresh vegetables, 6 varieties of canned seafood, canned chicken and 6 varieties of fresh cooked meats in amounts ranging from 0.23% to 0.005%.

It is possible that part of the superior flavor of young and of freshly harvested vegetables may be due to their higher glutamic acid content which decreased during short storage in the refrigerator. A high glutamic acid content may be one of the reasons mushrooms enhance the flavors of the many dishes to which they are added.

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